



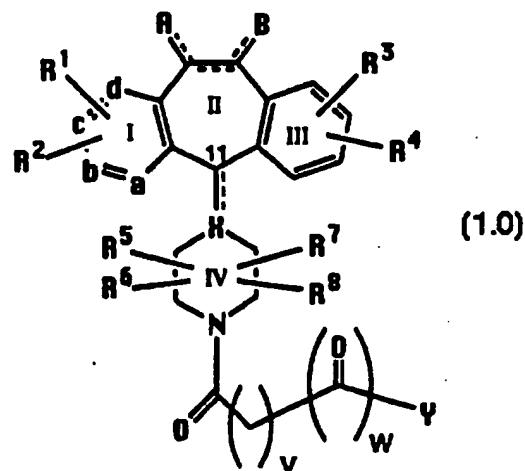
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(54) Title: TRICYCLIC ANTITUMOR COMPOUNDS BEING FARNESYL PROTEIN TRANSFERASE INHIBITORS

(57) Abstract

The invention relates to tricyclic compounds of formula (1.0) wherein R¹, R², R³, R⁴, R⁵, R⁶, R⁷, R⁸, Y, A, B, X, a, b, c, d, v, and w are as described herein. The compounds of formula (1.0) are useful for inhibiting tumor growth being farnesyl protein transferase inhibitors.



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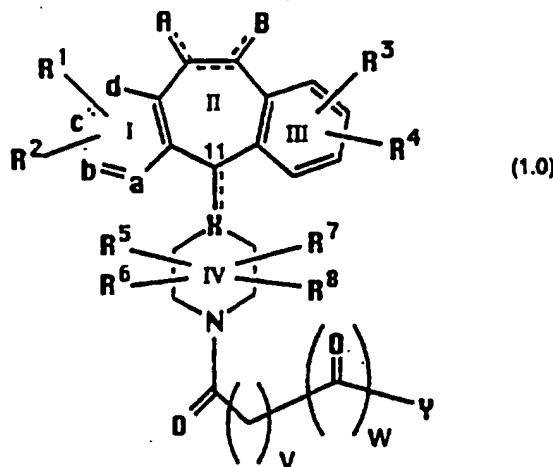
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TRICYCLIC ANTITUMOR COMPOUNDS BEING FARNESYL PROTEIN TRANSFERASE INHIBITORS

Summary of the Invention

5 This invention relates to compounds of the formula (1.0):

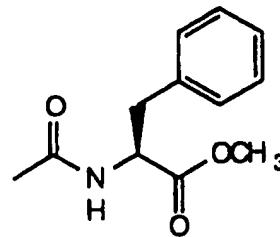


wherein:

X is N, CH, or C when the double bond is present at the C-11 position;

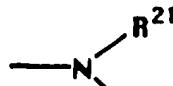
- 10 one of a, b, c and d represents N or NR⁹ wherein R⁹ is O⁻, -CH₃ or -(CH₂)_nCO₂H wherein n is 1 to 3, and the remaining a, b, c and d groups represent CR¹ or CR²; or
- each of a, b, c, and d are independently selected from CR¹ or CR²;
- 15 each R¹ and each R² is independently selected from H, halo, -CF₃, -OR¹⁰ (e.g., -OCH₃), -COR¹⁰, -SR¹⁰ (e.g., -SCH₃ and -SCH₂C₆H₅), -S(O)_tR¹¹ (wherein t is 0, 1 or 2, e.g., -SOCH₃ and -SO₂CH₃), -SCN, -N(R¹⁰)₂, -NR¹⁰R¹¹, -NO₂, -OC(O)R¹⁰, -CO₂R¹⁰, -OCO₂R¹¹, -CN, -NHC(O)R¹⁰, -NHSO₂R¹⁰, -CONHR¹⁰,
- 20 -CONHCH₂CH₂OH, -NR¹⁰COOR¹¹,

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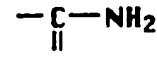
- SR¹¹C(O)OR¹¹ (e.g., -SCH₂CO₂CH₃), -SR¹¹N(R¹²)₂ wherein each R¹² is independently selected from H and -C(O)OR¹¹ (e.g.,
- S(CH₂)₂NHC(O)O-t-butyl and -S(CH₂)₂NH₂), benzotriazol-1-yloxy,
- 5 tetrazol-5-ylthio, or substituted tetrazol-5-ylthio (e.g., alkyl substituted tetrazol-5-ylthio such as 1-methyl-tetrazol-5-ylthio), alkynyl, alkenyl or alkyl, said alkyl or alkenyl group optionally being substituted with halo, -OR¹⁰ or -CO₂R¹⁰;
- R³ and R⁴ are the same or different and each independently represents H, any of the substituents of R¹ and R², or R³ and R⁴ taken together represent a saturated or unsaturated C₅-C₇ fused ring to the benzene ring (Ring III);
- 10 R⁵, R⁶, R⁷ and R⁸ each independently represents H, -CF₃, -COR¹⁰, alkyl or aryl, said alkyl or aryl optionally being substituted with
- 15 -OR¹⁰, -SR¹⁰, -S(O)_vR¹¹, -NR¹⁰COOR¹¹, -N(R¹⁰)₂, -NO₂, -COR¹⁰, -OCOR¹⁰, -OCO₂R¹¹, -CO₂R¹⁰, OPO₃R¹⁰, or R⁵ is combined with R⁶ to represent =O or =S and/or R⁷ is combined with R⁸ to represent =O or =S;
- R¹⁰ represents H, alkyl, aryl, or aralkyl (e.g., benzyl);
- 20 R¹¹ represents alkyl or aryl;
- the dotted line between carbon atoms 5 and 6 represents an optional double bond, such that when a double bond is present, A and B independently represent -R¹⁰, halo, -OR¹¹, -OCO₂R¹¹ or -OC(O)R¹⁰, and when no double bond is present between carbon atoms 5 and 6, A and B each independently represent H₂, -(OR¹¹)₂; H and halo, dihalo, alkyl and H, (alkyl)₂, -H and -OC(O)R¹⁰, H and -OR¹⁰, =O, aryl and H, =NOR¹⁰ or -O-(CH₂)_p-O- wherein p is 2, 3 or 4;
- v is 0 to 5;
- w is 0 or 1;

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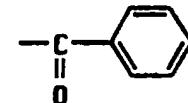
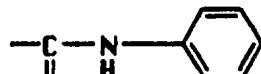
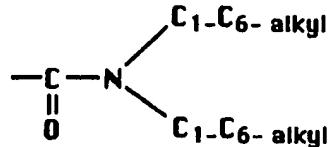


Y is R^{22} , $-\text{O}-\text{C}_1\text{-C}_6\text{-alkyl}$ or $-\text{OM}^+$, wherein M^+ is an alkali metal cation;

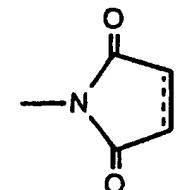
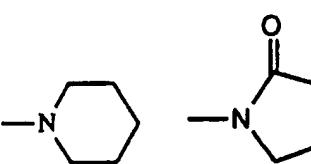
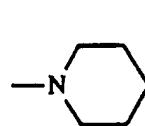
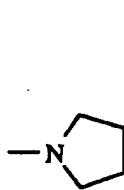
R^{21} and R^{22} are each independently H, $\text{C}_1\text{-C}_6$ alkyl, $-\text{CH}_2\text{CONH}_2$, phenyl, benzyl, $-\text{SO}_2\text{-(C}_1\text{-C}_6\text{-alkyl)}$, $-\text{NH-phenyl}$, acyl, C_3 -



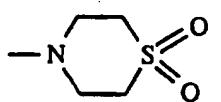
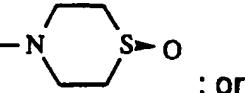
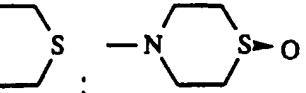
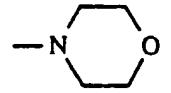
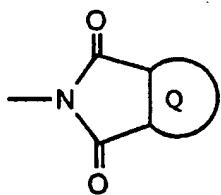
5 C_6 cycloalkyl, pyridyl, chloro-phenyl,



or R^{21} and R^{22} taken together with the nitrogen to which they are attached form



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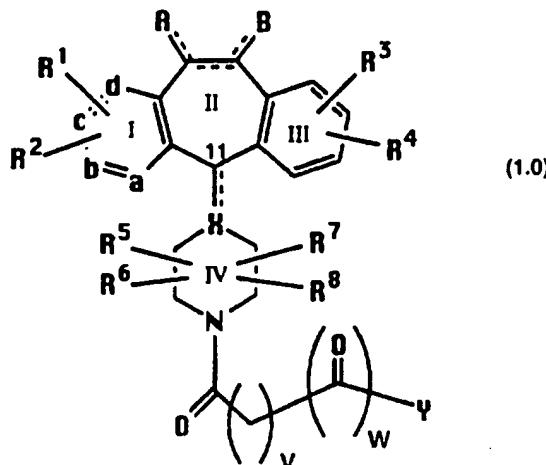
a dashed line means an optional chemical bond;

wherein Q is benzene, or a heterocyclic ring such as pyridine, pyrazine, or thiophene;

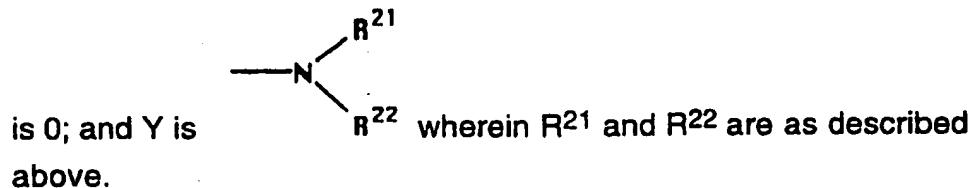
15 or a pharmaceutically acceptable salt thereof.

Preferred among compounds of the invention are compounds of the formula (1.0)

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wherein R¹, R², X, A, B, a, b, c, d, v are as described above, v is 0 to 4; w



5 Also preferred are compounds of formula (1.0) wherein a
is N; R⁵, R⁶, R⁷ and R⁸ are all H; and R¹, R², and R³ are each
independently selected from the group consisting of H or halo.

Also preferred are compounds of formula (1.0) wherein R¹ is H; and R² is Br; and R³ and R⁴ are each independently selected from the group consisting of Br and Cl.

Also preferred are compounds of formula (1.0) wherein X is

Also preferred are compounds of any one of formula (1.0) wherein R³ is Cl; and R⁴ is Br.

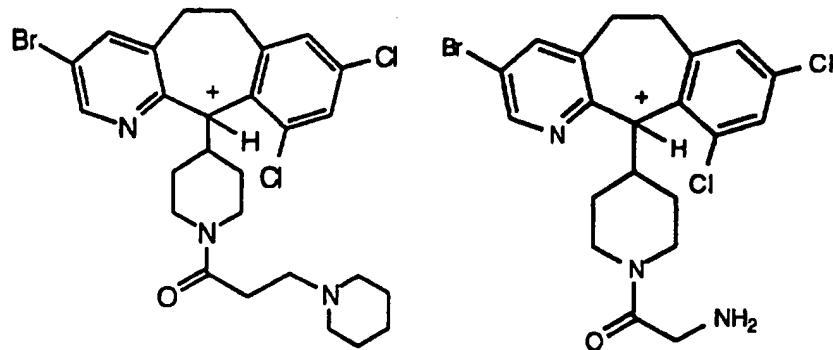
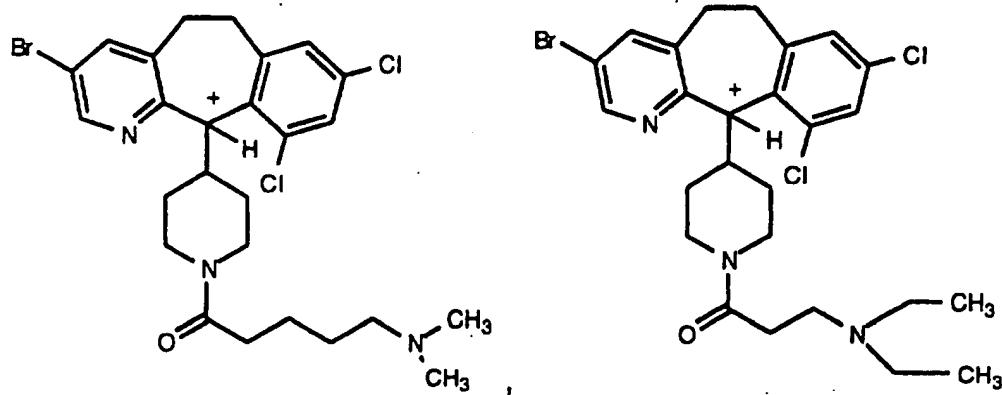
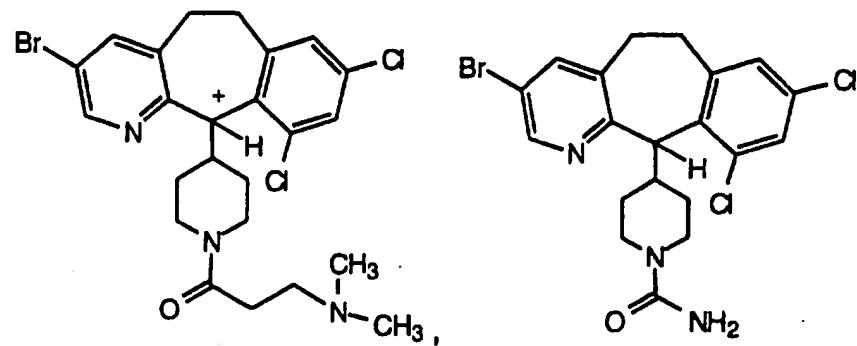
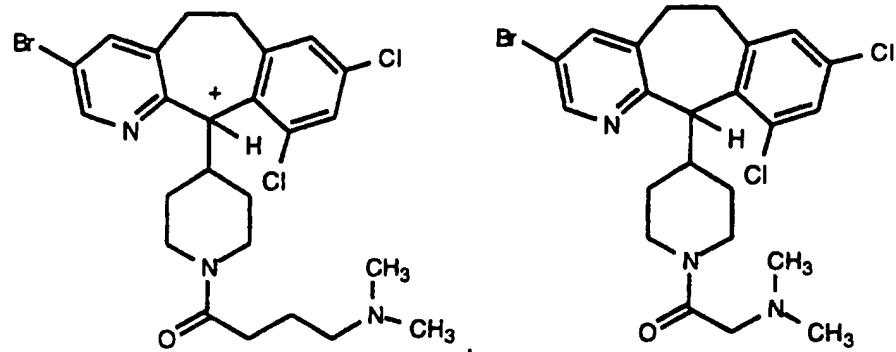
15 Also preferred are compounds of formula (1.0) wherein a is N or NO⁻, and R⁵, R⁶, R⁷ and R⁸ are all H; and R¹, R², R³ and R⁴ are each independently selected from the group consisting of H or halo.

Also preferred are compounds of formula (1.0) wherein A and B are each H₂; b and d are preferably CH.

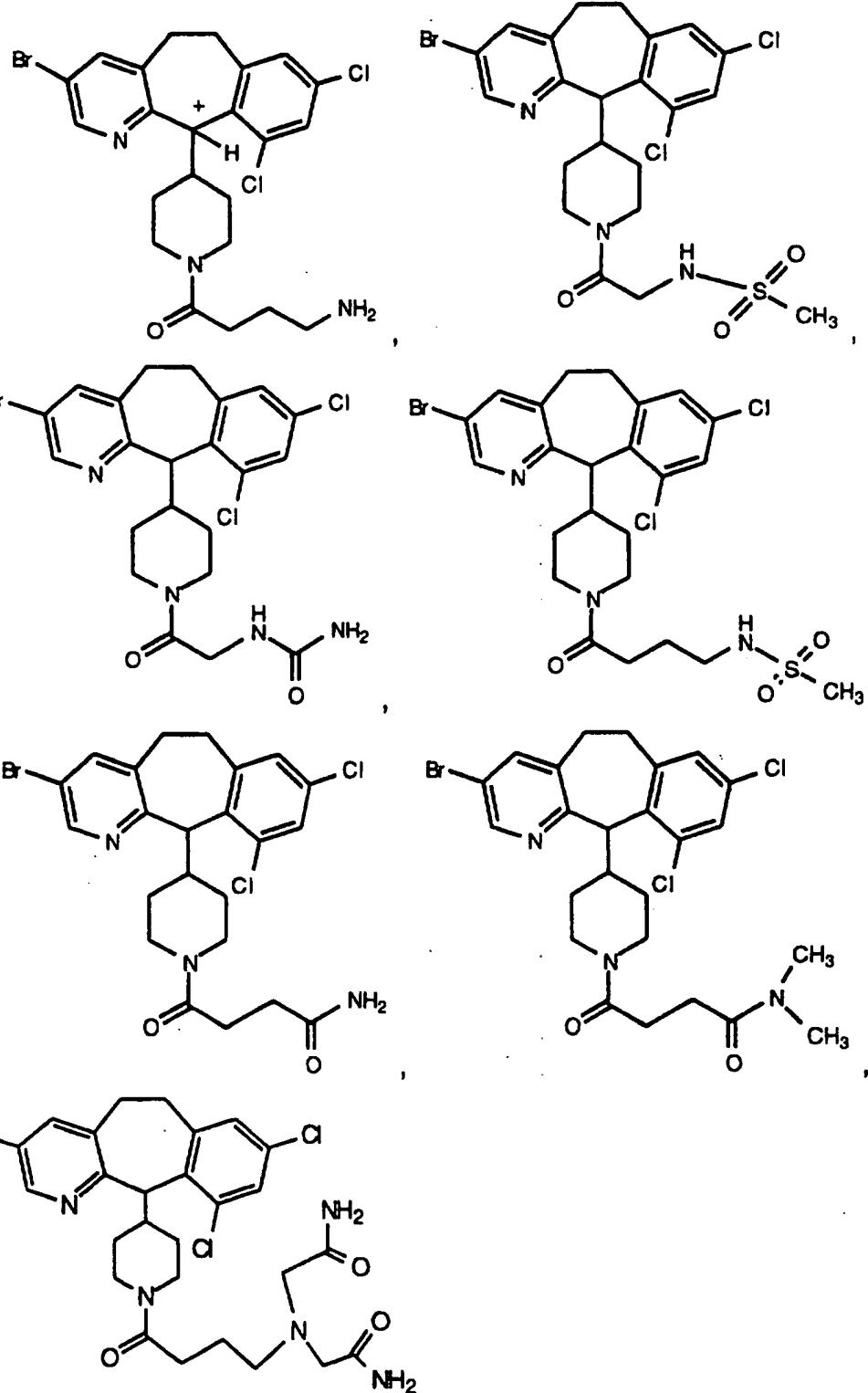
20 Another group of preferred compounds is that wherein w is 1; v is 0-5; R¹ is H; R² is Br; R³ and R⁴ are independently Cl and Br; R⁵-R⁸ are each H; X is CH; and Y is -O-C₁-C₆ alkyl, NH₂ or -OM⁺.

Exemplary of compounds of the invention are:

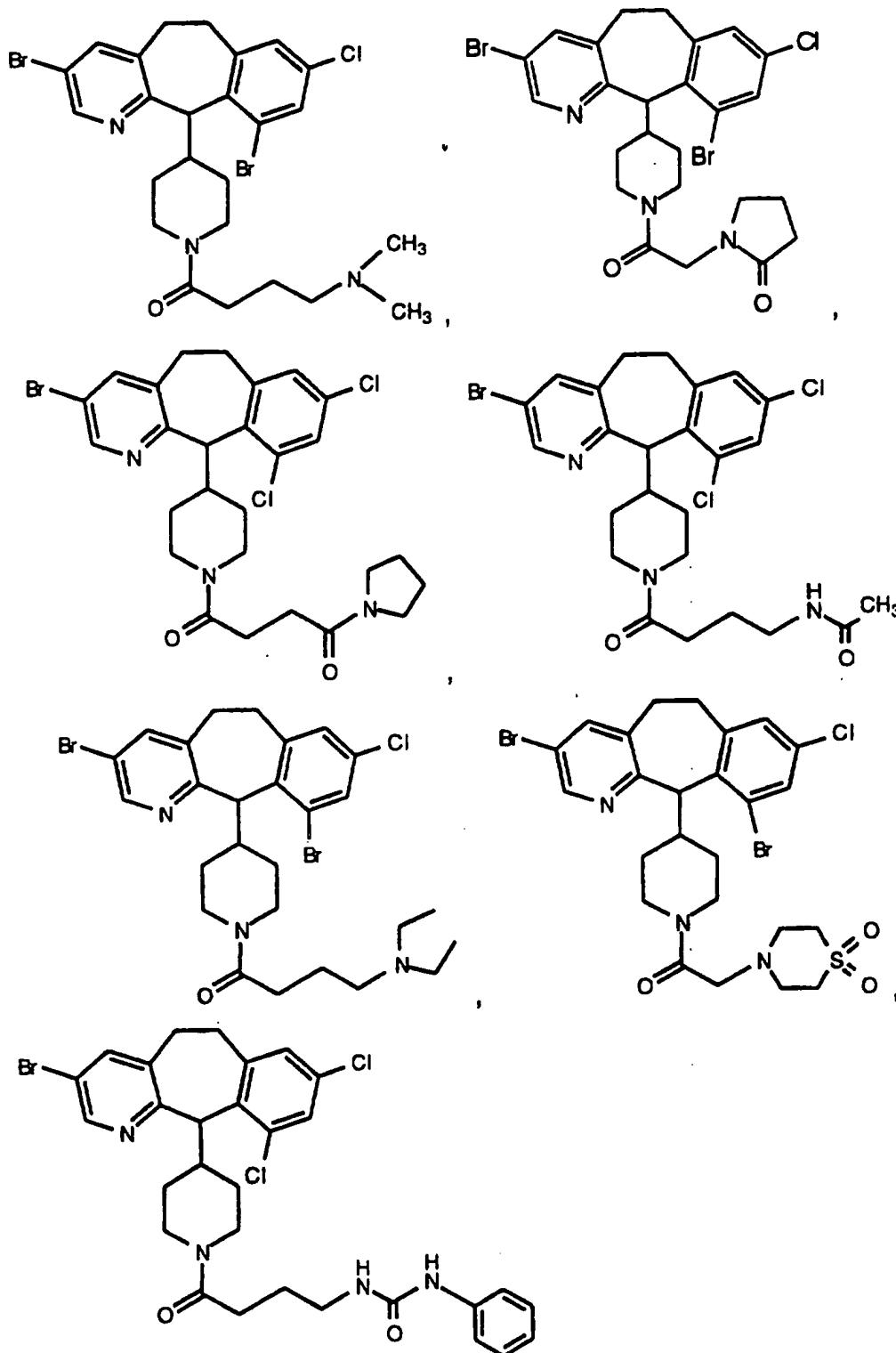
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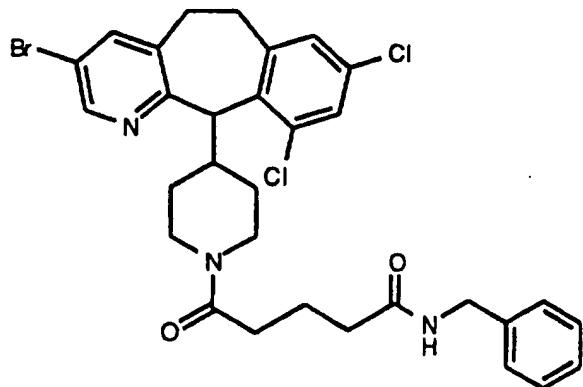
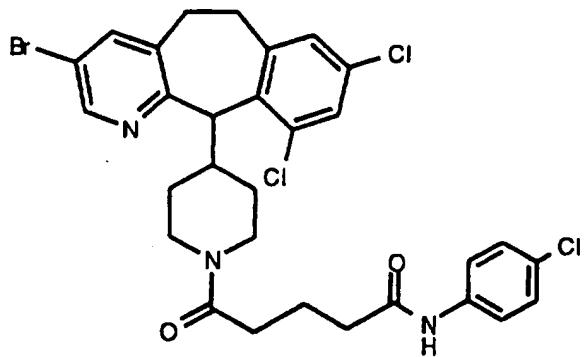
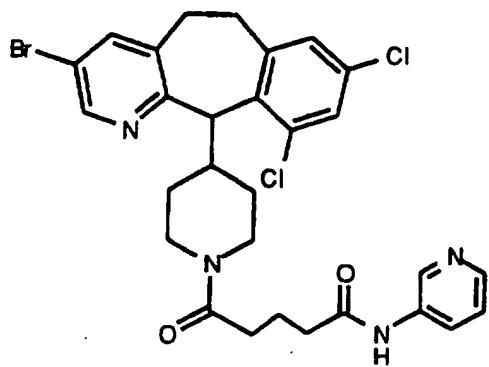
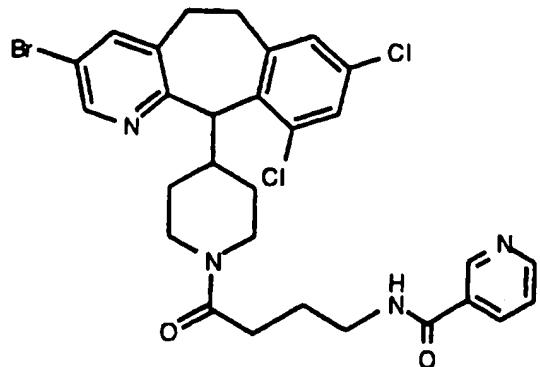
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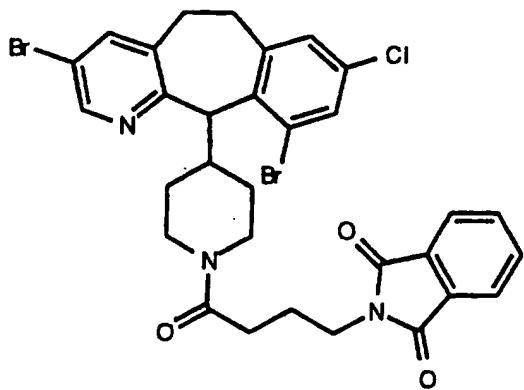
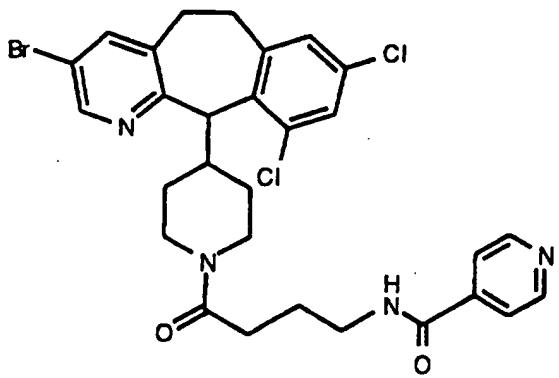
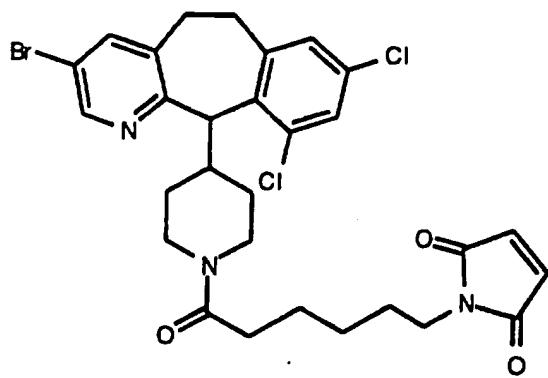
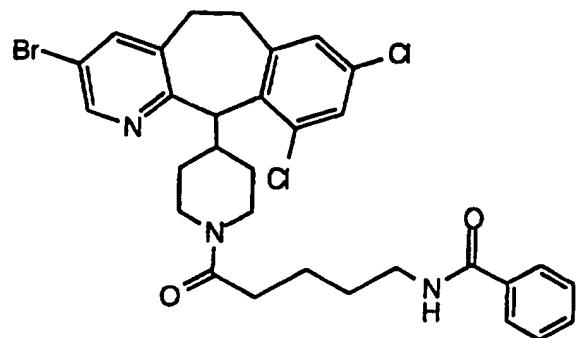
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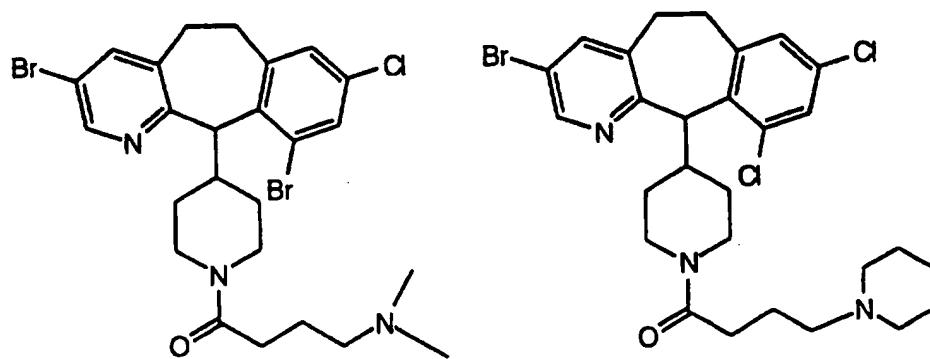
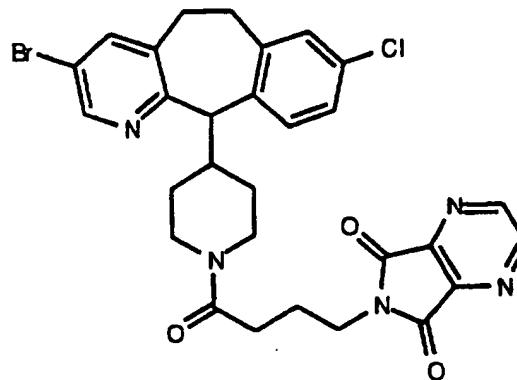
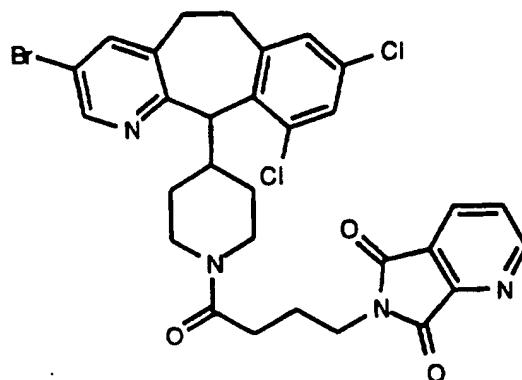
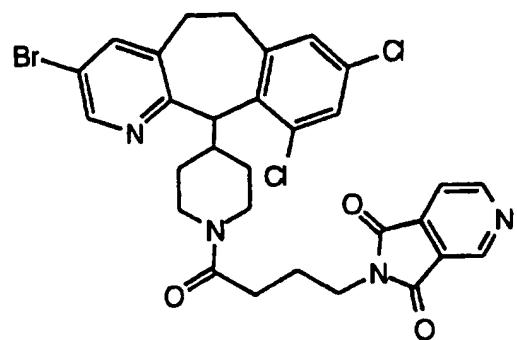
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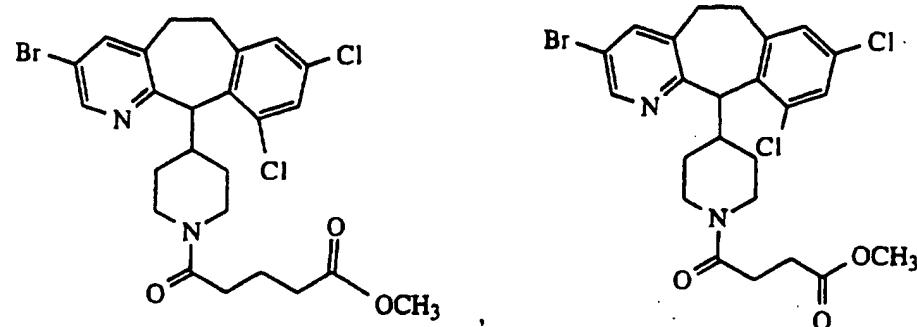
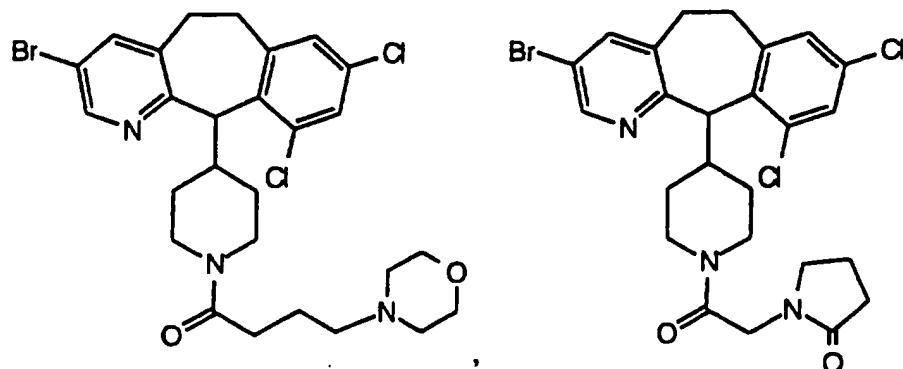
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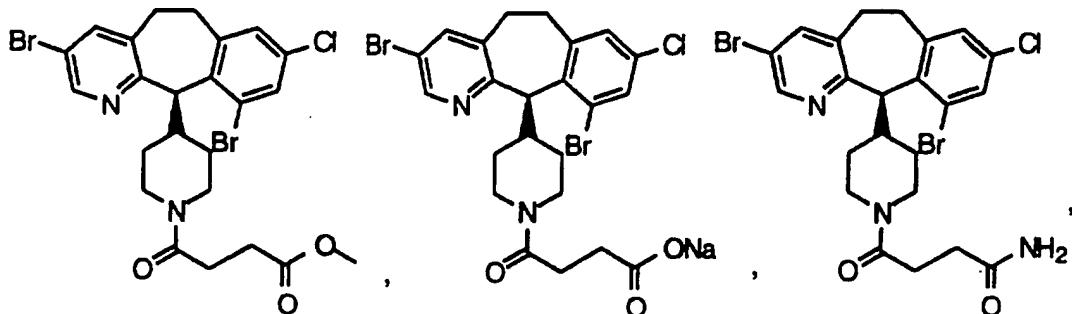
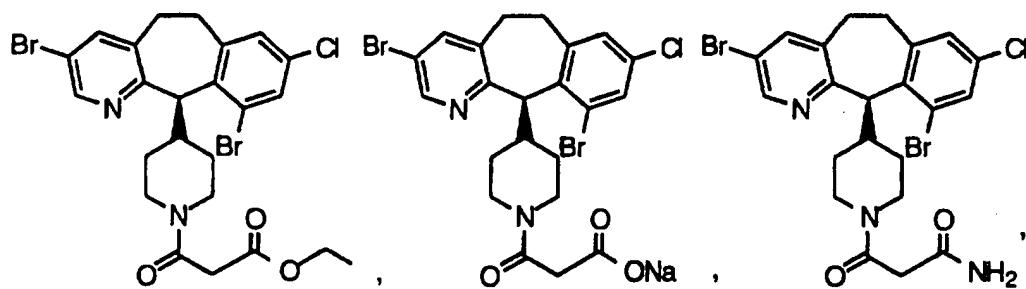
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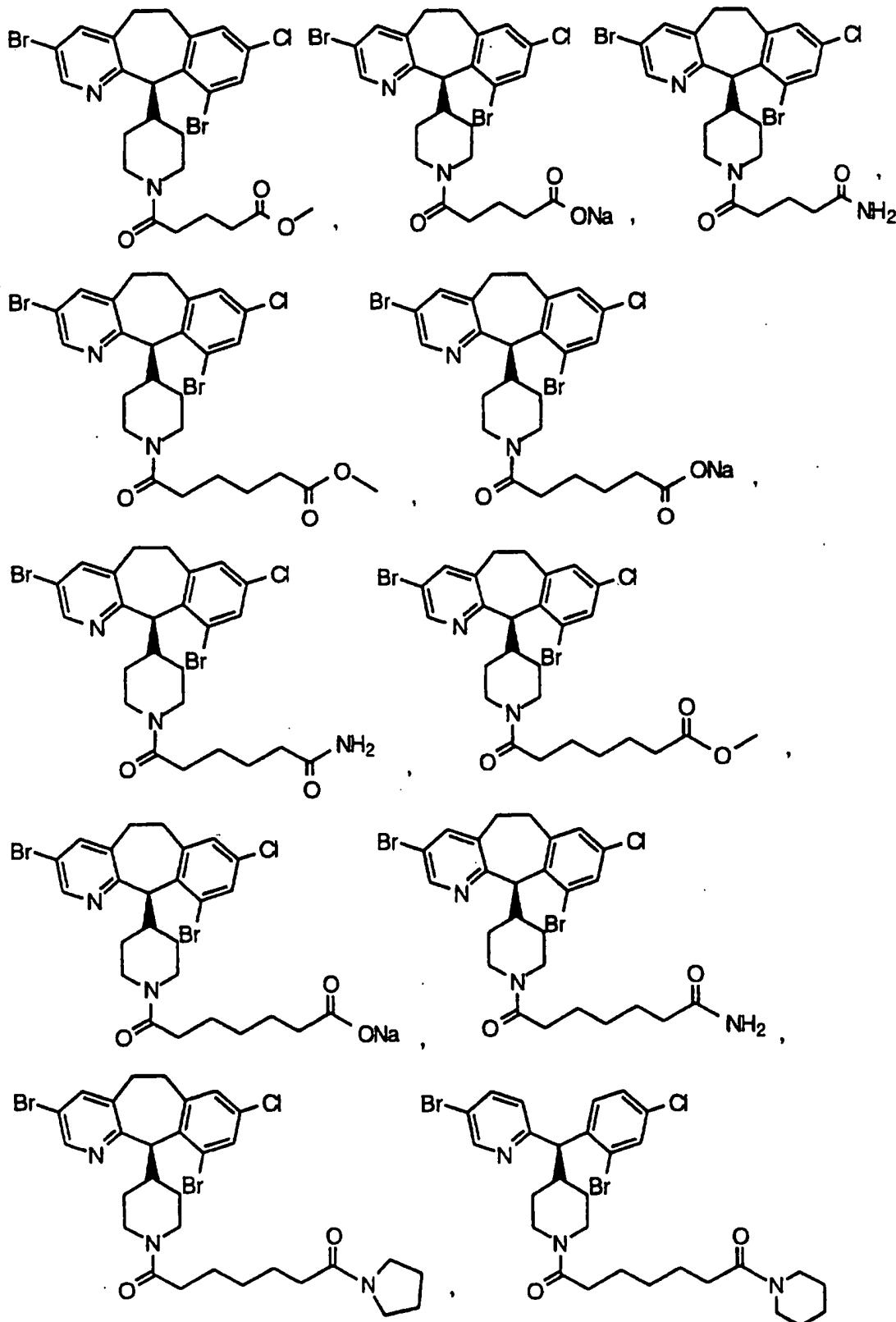
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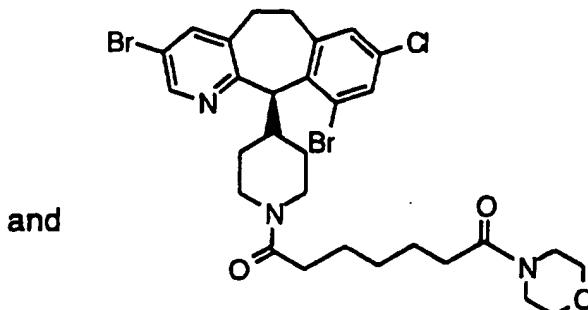
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The compounds of formula (1.0) are useful as farnesyl protein transferase inhibitors. Accordingly, the compounds of formula (1.0) are useful for inhibiting tumor growth. Examples of tumors which may be

- 5 inhibited include, but are not limited to, breast cancer, prostatic cancer, lung cancer (e.g., lung adenocarcinoma), pancreatic cancers (e.g., pancreatic carcinoma such as, for example, exocrine pancreatic carcinoma), colon cancers (e.g., colorectal carcinomas, such as, for example, colon adenocarcinoma and colon adenoma), myeloid
- 10 leukemias (for example, acute myelogenous leukemia (AML)), thyroid follicular cancer, myelodysplastic syndrome (MDS), bladder carcinoma and epidermal carcinoma.

The invention also relates to pharmaceutical compositions for treating tumors which comprise a compound of formula (1.0) and a pharmaceutically acceptable carrier material.

The invention also relates to a method for treating tumors which comprises administering an anti-tumor effective amount of a compound of formula (1.0).

20 **DETAILED DESCRIPTION OF THE INVENTION**

The compounds of formula (1.0) can exist in unsolvated as well as solvated forms, including hydrated forms, e.g. the hemihydrate. In general, the solvated forms, with pharmaceutically acceptable solvents such as water, ethanol, and the like are equivalent to the unsolvated forms for the purposes of the invention.

Certain compounds of the invention can exist in stereoisomeric form. All such isomeric forms and mixtures thereof are within the scope of the present invention. Unless otherwise indicated, the methods of preparation disclosed herein may result in product distributions which

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include all possible structural isomers, although it is understood that physiological response may vary according to stereochemical structure. The isomers may be separated by conventional means such as fractional crystallization or HPLC (high performance liquid chromatography).

The compounds of formula (1.0) form pharmaceutically acceptable salts. The preferred pharmaceutically acceptable salts are nontoxic acid addition salts formed by adding to a suitable compound of the invention about a stoichiometric amount of a mineral acid , such as HCl, HBr, H₂SO₄ or H₃PO₄ or of an organic acid such as acetic, propionic, valeric, oleic, palmitic, stearic, lauric, benzoic, lactic, para-toluenesulfonic, methane sulfonic, citric, maleic, fumaric, succinic and the like, respectively.

When utilized herein and in the appended claims, the following terms, unless otherwise specified have the following meanings:

alkyl (including the alkyl portions of alkoxy, alkylamino and dialkylamino) represents straight and branched carbon chains and contains from one to twenty carbon atoms, preferably one to six carbon atoms;

acyl represents a moiety of the formula  R , wherein R is C₁-C₆ alkyl, phenyl, pyridyl, chlorophenyl, as described above ;

alkanediyl represents a divalent, straight or branched hydrocarbon chain having from 1 to 20 carbon atoms, preferably 1 to 6 carbon atoms, the two available bonds being from the same or different carbon atoms thereof, e.g., methylene, ethylene, ethyldene, -CH₂CH₂CH₂-, -CH₂CHCH₃, -CHCH₂CH₃, etc.

cycloalkyl represents saturated carbocyclic rings branched or unbranched of from 3 to 20 carbon atoms, preferably 3 to 7 carbon atoms;

chlorophenyl represents a phenyl moiety wherein one of the hydrogens is replaced by a chlorine;

alkenyl represents straight and branched carbon chains having at least one carbon to carbon double bond and containing from 2 to 12

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carbon atoms, preferably from 2 to 6 carbon atoms and most preferably from 3 to 6 carbon atoms;

alkynyl represents straight and branched carbon chains having at least one carbon to carbon triple bond and containing from 2 to 12

5 carbon atoms, preferably from 2 to 6 carbon atoms;

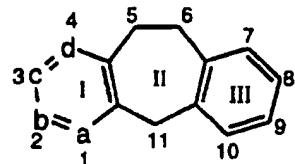
aryl (including the aryl portion of aryloxy and aralkyl) represents a carbocyclic group containing from 6 to 15 carbon atoms and having at least one aromatic ring (e.g., aryl is a phenyl ring), with all available substitutable carbon atoms of the carbocyclic group being intended as

10 possible points of attachment, said carbocyclic group being optionally substituted (e.g., 1 to 3) with one or more of halo, alkyl, hydroxy, alkoxy, phenoxy, CF₃, amino, alkylamino, dialkylamino, -COOR¹⁰ or -NO₂;

M⁺ is an alkali metal cation, preferably a sodium or lithium cation;

15 and halo represents fluoro, chloro, bromo and iodo.

Reference to the position of the substituents R¹, R², R³, and R⁴ is based on the numbered ring structure:



For example, R¹ can be at the C-4 position and R² can be at the C-2 or 20 C-3 position. Also, for example, R³ can be at the C-8 position and R⁴ can be at the C-10 position.

When the bond from the IV ring to the C-11 carbon is a single bond, all stereoisomers are included within formula (1.0), that is, racemates, R-isomers, and S-isomers.

25 The compounds of the invention can be prepared according to the following methods.

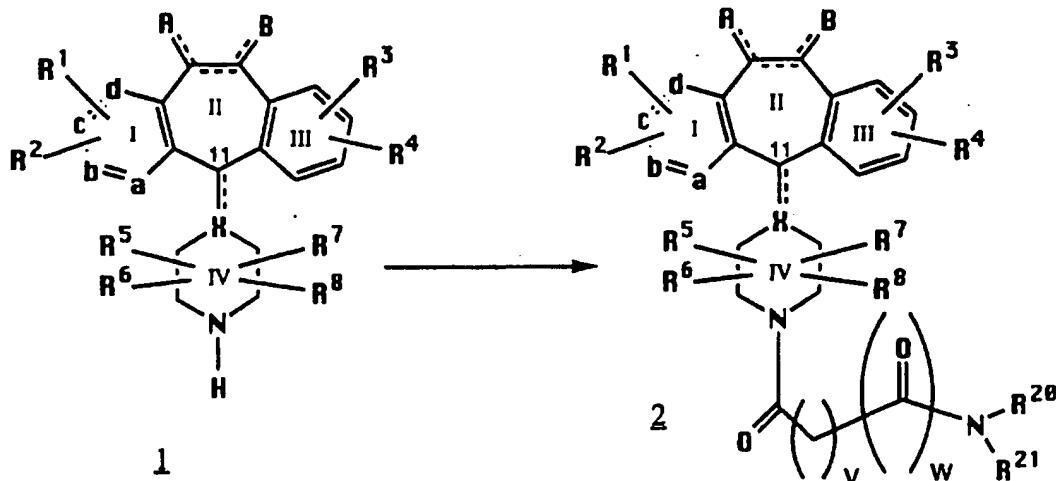
The compounds of the invention are prepared as shown by reaction schemes 1, 2, and 3. Other conventional techniques such as ester hydrolysis and cleavage of protecting groups may be employed in 30 the procedures to prepare the compounds of the invention.

Specifically, Scheme 1 depicts the synthesis of amides by the treatment of amine 1 with the appropriate carboxylic acid in the

presence of a coupling agent such as DEC. Scheme 1A depicts the preparation of compounds of formula I wherein w is 1: esters, wherein Y is -O-alkyl, are prepared by reaction of an amine of formula 1 with a diacid monoester of formula 10 using standard coupling reagents, e.g.,

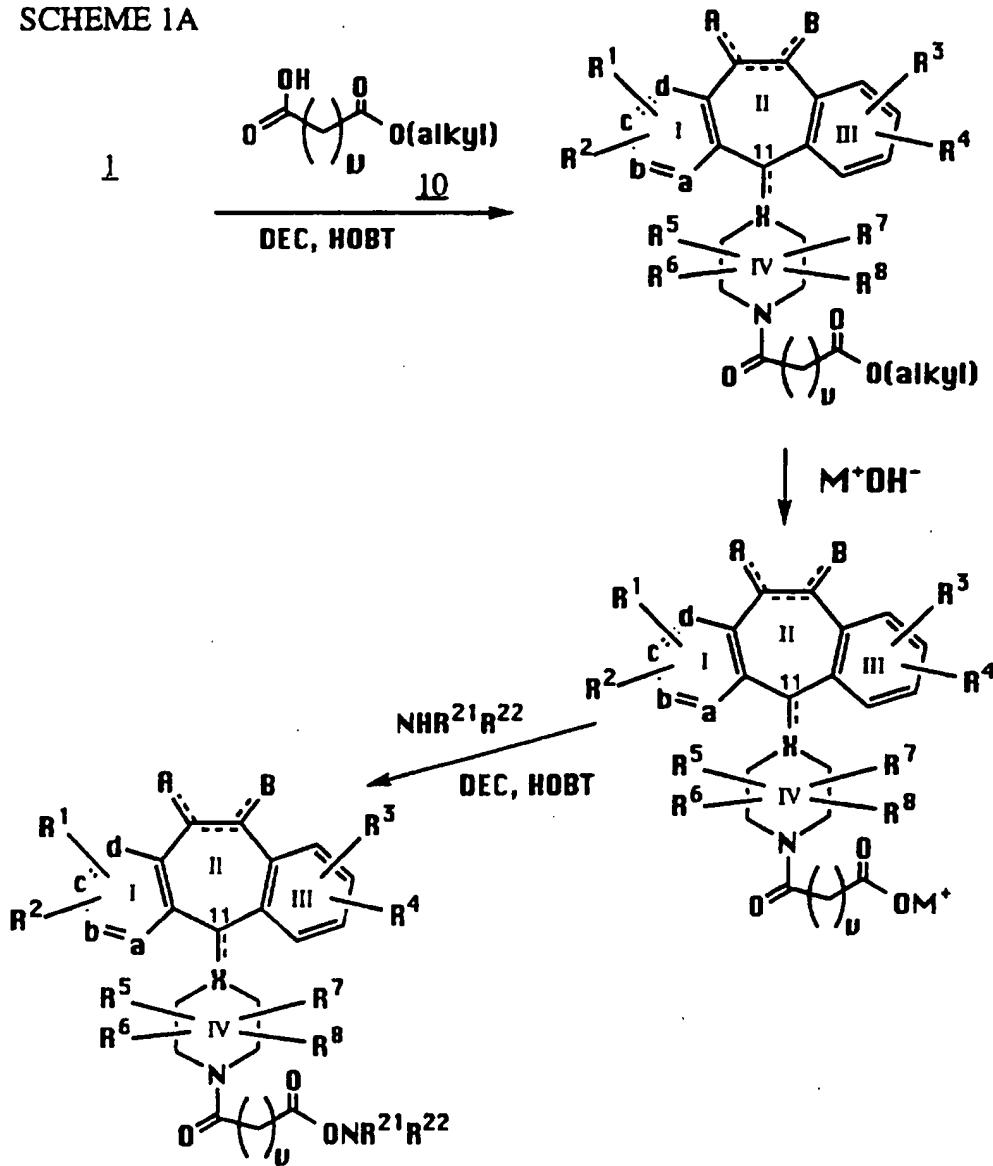
- 5 DEC and HOBT; the resultant ester can be hydrolyzed to an acid and converted to an alkali metal salt by conventional means, e.g., the sodium salt can be prepared by dissolving the ester in alcohol and treating with NaOH. The salt can then be reacted with an amine, again using standard coupling procedures, e.g., DEC and HOBT, to obtain amides of
- 10 formula I (i.e., compounds wherein Y is -NR²¹R²²).

SCHEME 1



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SCHEME 1A



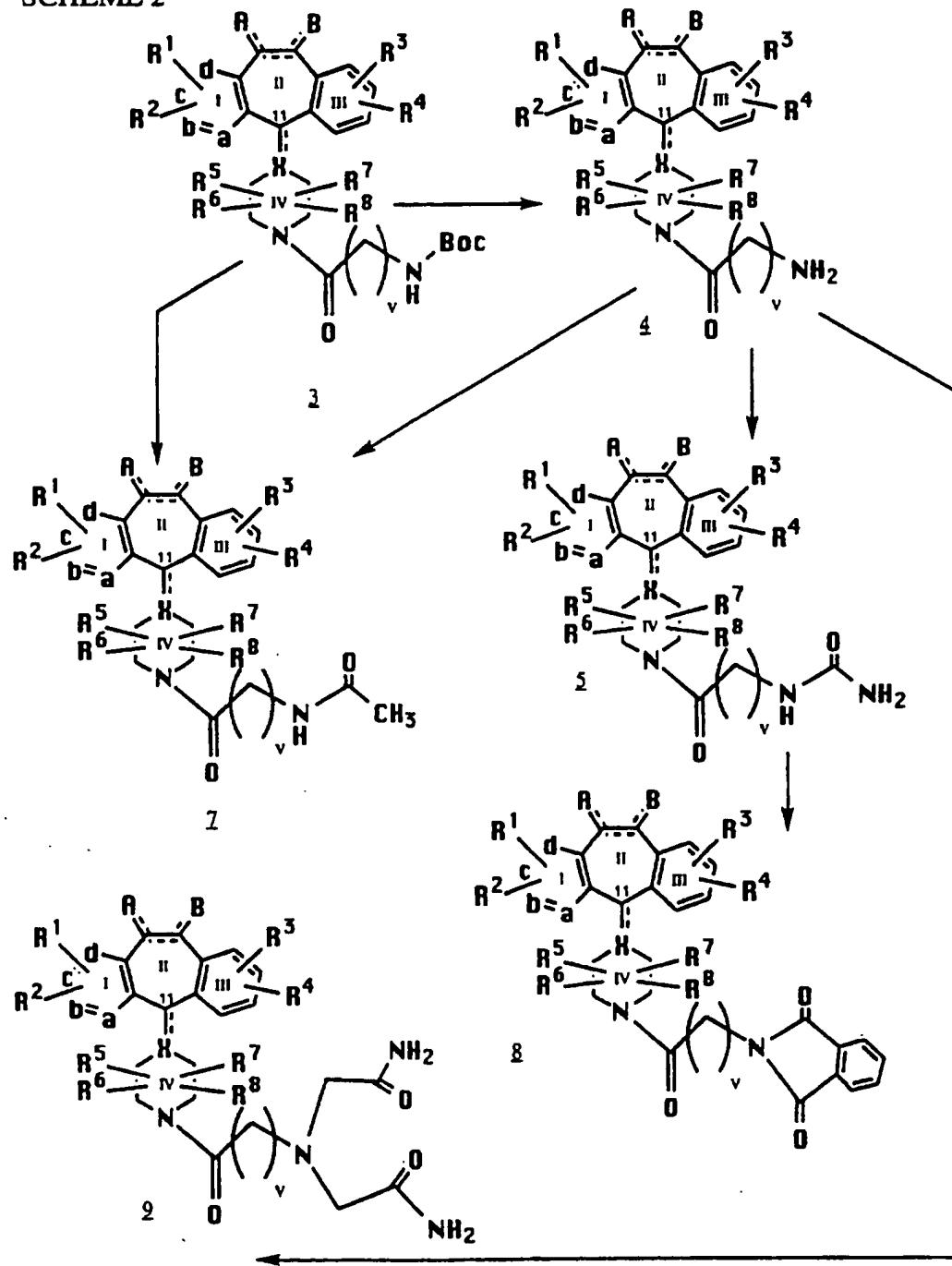
Scheme 2 depicts preparation of ureas of formula 5, acetamides of formula 7 and diamides of formula 8. In Scheme 2, the N-BOC protecting group is removed using TFA/CH₂Cl₂ or dioxane-HCl.

5 The resulting amine 4 is treated with trimethylsilyl isocyanate in TFA to give ureas 5. Treatment of compounds of formula 4 with either acetyl chloride or acetic anhydride provides the acetamides of formula 7. Treatment of 5 with carboxylic anhydride in dimethylformamide at a temperature between about 40°C to 50°C and then treating the resulting adduct with Ac₂O and heating between about 85°C-95°C gives

10 diamides of the formula 8.

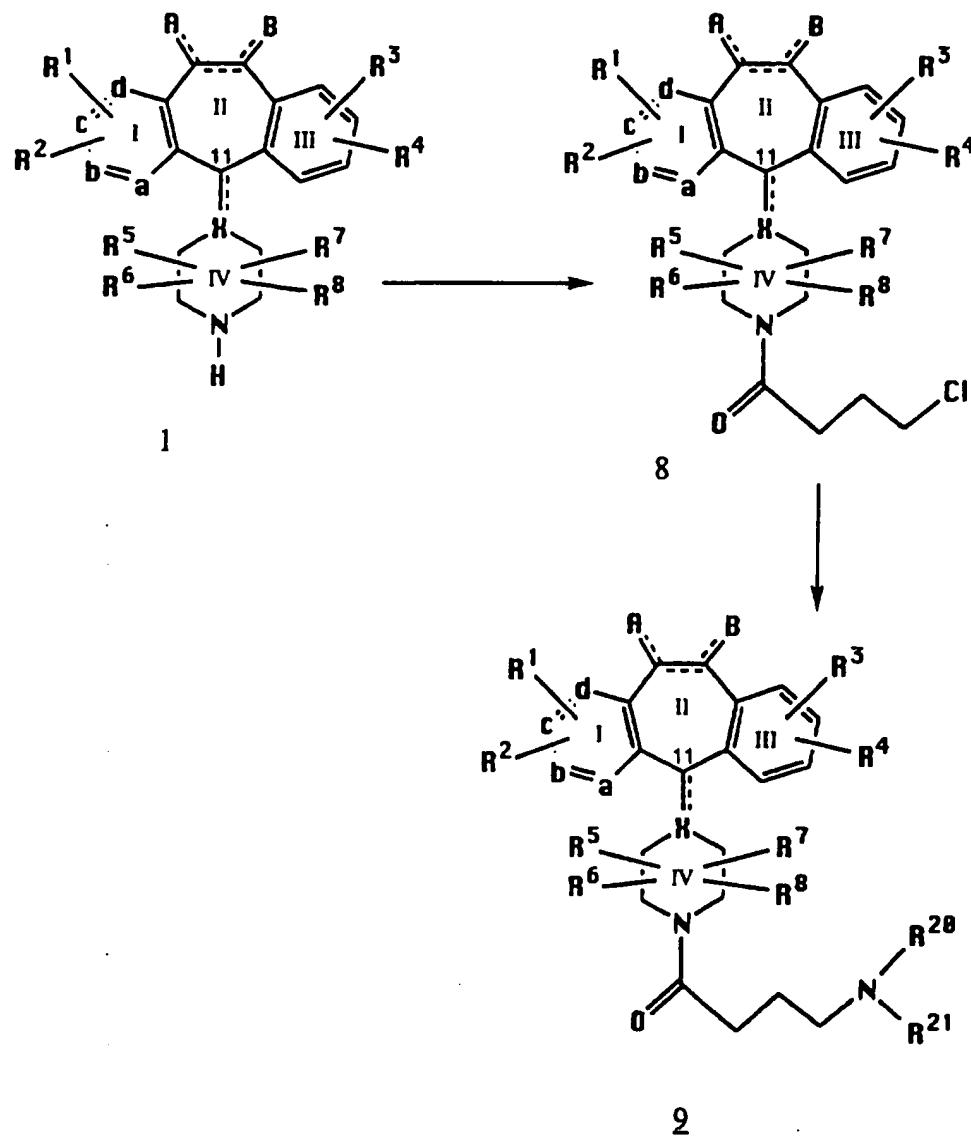
-18-

SCHEME 2



Scheme 3 shows the reaction of amine 1 with 4-chlorobutyl chloride to give an amide of the formula 8. Treatment of an amide of 5 formula 8 with an amine affords the 4-amino substituted analogs of formula 9.

Scheme 3

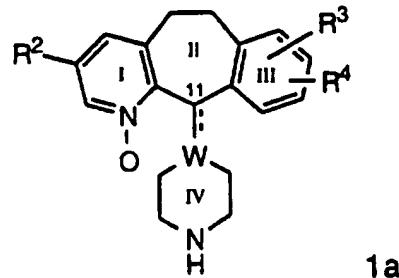


Starting materials used in the preparation of compounds of the invention are either known, can be prepared according to known methods, or can be prepared by methods that are analogous to known methods.

Compounds of formula I comprising a pyridyl N-oxide in ring I of the tricyclic portion can be prepared by procedures well known in the art. For example, the amine compound of formula 1 can be reacted with

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MCPBA in a suitable organic solvent, e.g., CH₂Cl₂ (usually anhydrous), at a suitable temperature, to obtain an N-oxide of formula 1a



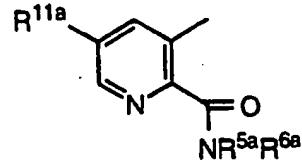
Generally, the organic solvent solution of formula 1 is cooled to

5 about 0°C before the MCPBA is added. The reaction is then allowed to warm to room temperature during the reaction period. The desired product can be recovered by standard separation means, for example, the reaction mixture can be washed with an aqueous solution of a suitable base, e.g., saturated NaHCO₃ or NaOH (e.g., 1 N NaOH), and

10 then dried over anhydrous MgSO₄. The solution containing the product can be concentrated in vacuo, and the product can be purified by standard means, e.g., by chromatography using silica gel (e.g., flash column chromatography).

Compounds of formula 1 are prepared by methods known in the art, for example by methods disclosed in WO 95/10516, in U.S. 15 5,151,423 and those described below. Compounds of formula 1 wherein the C-3 position of the pyridine ring in the tricyclic structure is substituted by bromo can also be prepared by a procedure comprising the following steps:

20 (a) reacting an amide of the formula

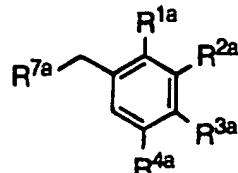


wherein R^{11a} is Br, R^{5a} is hydrogen and R^{6a} is C₁-C₆ alkyl, aryl or heteroaryl; R^{5a} is C₁-C₆ alkyl, aryl or heteroaryl and R^{6a} is hydrogen; R^{5a} and R^{6a} are independently selected from the group consisting of C₁-25 C₆ alkyl and aryl; or R^{5a} and R^{6a}, together with the nitrogen to which they are attached, form a ring comprising 4 to 6 carbon atoms or comprising 3 to 5 carbon atoms and one hetero moiety selected from the

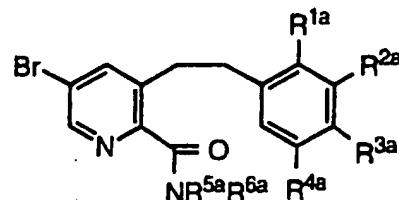
-21-

group consisting of -O- and -NR^{9a}-, wherein R^{9a} is H, C₁-C₆ alkyl or phenyl;

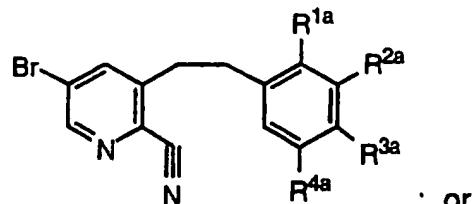
with a compound of the formula



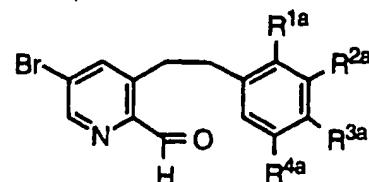
5 wherein R^{1a}, R^{2a}, R^{3a} and R^{4a} are independently selected from the group consisting of hydrogen and halo and R^{7a} is Cl or Br, in the presence of a strong base to obtain a compound of the formula



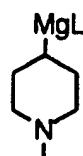
(b) reacting a compound of step (a) with
10 (i) POCl₃ to obtain a cyano compound of the formula



(ii) DIBALH to obtain an aldehyde of the formula

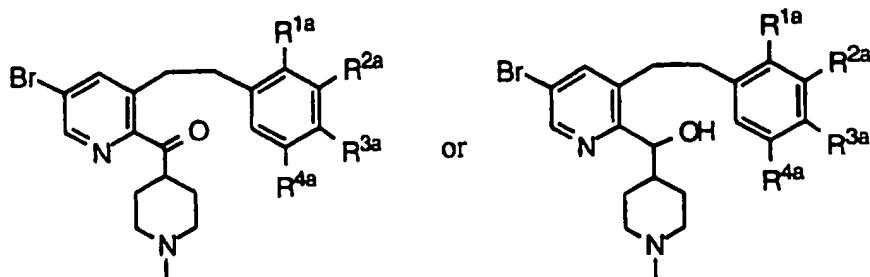


15 (c) reacting the cyano compound or the aldehyde with a piperidine derivative of the formula



wherein L is a leaving group selected from the group consisting of Cl and Br, to obtain an aldehyde or an alcohol of the formula below, respectively:

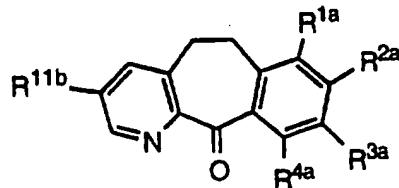
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(d)(i) cyclizing the aldehyde with $\text{CF}_3\text{SO}_3\text{H}$ to obtain a compound of formula II wherein the dotted line represents a double bond; or

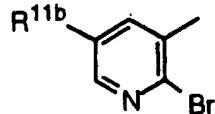
5 (d)(ii) cyclizing the alcohol with polyphosphoric acid to obtain a compound of formula II wherein the dotted line represents a single bond.

Methods for preparing compounds of formula 1 disclosed in WO 95/10516, U.S. 5,151,423 and described below employ a tricyclic ketone intermediate. Such intermediates of the formula

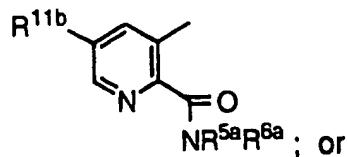


10 wherein R^{11b} , R^{1a} , R^{2a} , R^{3a} and R^{4a} are independently selected from the group consisting of hydrogen and halo, can be prepared by the following process comprising :

(a) reacting a compound of the formula

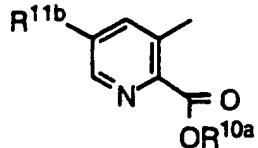


15 (i) with an amine of the formula $\text{NHR}^{5a}\text{R}^{6a}$, wherein R^{5a} and R^{6a} are as defined in the process above; in the presence of a palladium catalyst and carbon monoxide to obtain an amide of the formula:



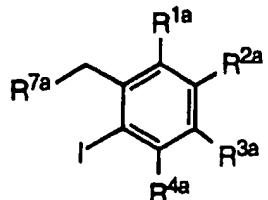
20 (ii) with an alcohol of the formula R^{10a}OH , wherein R^{10a} is C₁-C₆ lower alkyl or C₃-C₆ cycloalkyl, in the presence of a palladium catalyst and carbon monoxide to obtain the ester of the formula

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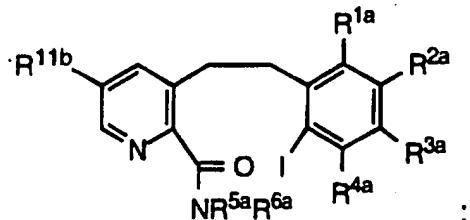


followed by reacting the ester with an amine of formula NHR^{5a}R^{6a} to obtain the amide;

5 (b) reacting the amide with an iodo-substituted benzyl compound of the formula



wherein R^{1a}, R^{2a}, R^{3a}, R^{4a} and R^{7a} are as defined above, in the presence of a strong base to obtain a compound of the formula

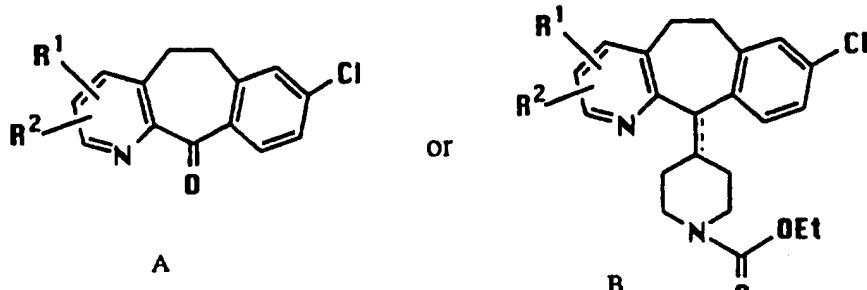


10 (c) cyclizing a compound of step (b) with a reagent of the formula R^{8a}MgL, wherein R^{8a} is C₁-C₈ alkyl, aryl or heteroaryl and L is Br or Cl, provided that prior to cyclization, compounds wherein R^{5a} or R^{6a} is hydrogen are reacted with a suitable N-protecting group.

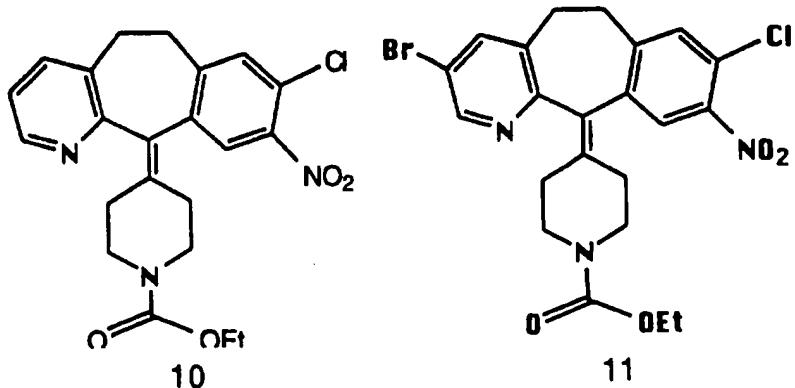
15 (+)-Isomers of compounds of formula 1 wherein X is CH can be prepared with high enantioselectivity by using a process comprising enzyme catalyzed transesterification. Preferably, a racemic compound of formula 1, wherein X is C, the double bond is present and a substituent other than H is present at the 10-position on ring III, is reacted with an enzyme such as Toyobo LIP-300 and an acylating agent such as trifluoroethyl isobutyrate; the resultant (+)-amide is then hydrolyzed, for example by refluxing with an acid such as H₂SO₄, to obtain the corresponding optically enriched (+)-isomer. Alternatively, a racemic compound of formula 1, wherein X is C, the double bond is present and a substituent other than H is present at the 10-position on ring III, is first reduced to the corresponding racemic compound of

formula 1 wherein X is CH and then treated with the enzyme (Toyobo LIP-300) and acylating agent as described above to obtain the (+)-amide, which is hydrolyzed to obtain the optically enriched (+)-isomer.

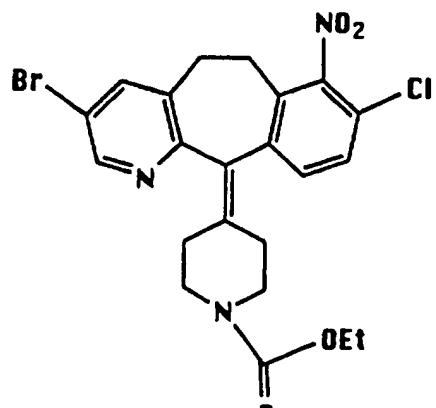
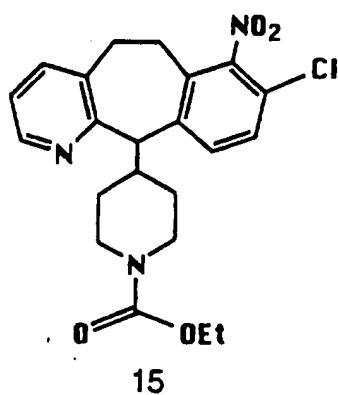
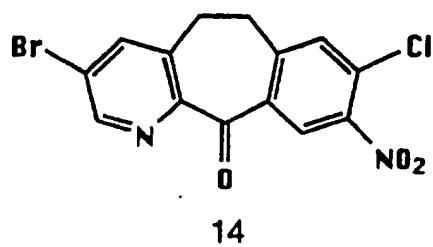
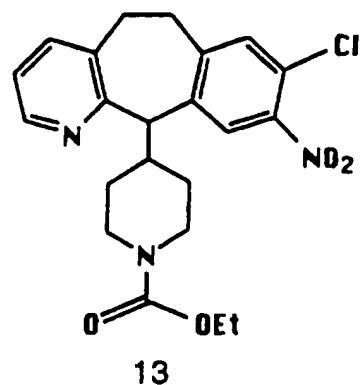
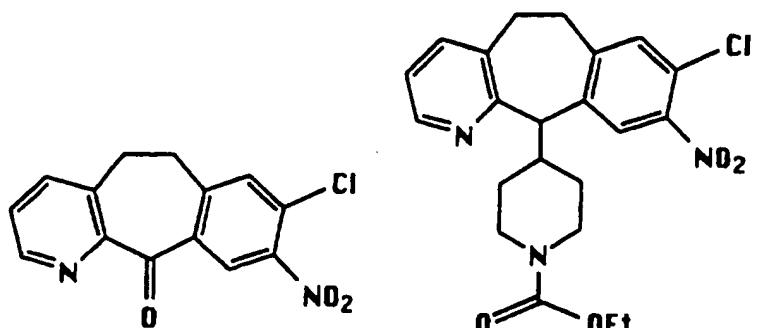
Nitration Process



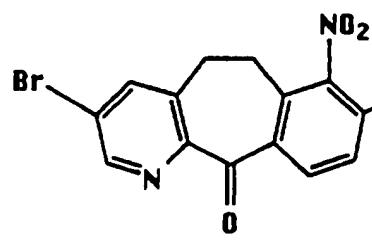
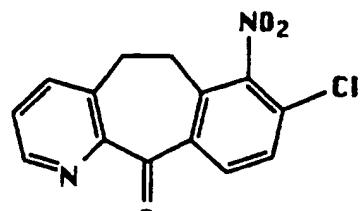
5 A process for producing compounds of the formulas 10 through
18 shown below. These compounds are useful as intermediates in the
preparation of compounds of formula (1.0) of the invention. The process
involves suspending one molar equivalent of either formula A or B
10 above in an appropriate aqueous acid such as concentrated sulfuric
acid and then cooling the reaction mixture to -20 to 40°C and then
adding 1.1 molar equivalent of KNO₃ at the same temperature. The
reaction mixture is stirred at that temperature for 1 hour and then allowed
to warm up to room temperature over a period of 10 to 16 hours. The
15 reaction mixture is then poured onto ice and basified with an
appropriate base such as concentrated ammonium hydroxide or 50%
aqueous NaOH. It is extracted with an appropriate solvent such as
CH₂Cl₂ and desired compounds are obtained either by recrystallization
or column chromatography.



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Biological activity of the compounds of the invention as farnesyl protein transferase inhibitors can be demonstrated by the assays below.

ASSAYS

1. In vitro enzyme assays: Inhibition of farnesyl protein transferase and geranylgeranyl protein transferase.
 - 5 Farnesyl protein transferase (FPT) was partially purified from rat brain by ammonium sulfate fractionation followed by Q-Sepharose (Pharmacia, Inc.) anion exchange chromatography essentially as described by Yokoyama et al (Yokoyama, K., et al., (1991), A protein
 - 10 geranylgeranyltransferase from bovine brain: Implications for protein prenylation specificity, Proc. Natl. Acad. Sci USA 88: 5302-5306, the disclosure of which is incorporated herein by reference thereto). Human farnesyl protein transferase was also expressed in *E. coli*, using cDNA clones encoding both the a and b subunits. The methods used
 - 15 were similar to those published (Omer, C. et al., (1993), Characterization of recombinant human farnesyl protein transferase: Cloning, expression, farnesyl diphosphate binding, and functional homology with yeast prenyl-protein transferases, Biochemistry 32:5167-5176). Human farnesyl protein transferase was partially-purified from the soluble
 - 20 protein fraction of *E. coli* as described above. The tricyclic farnesyl protein transferase inhibitors disclosed herein inhibited both human and rat enzyme with similar potencies. Two forms of val¹²-Ha-Ras protein were prepared as substrates for these enzymes, differing in their carboxy terminal sequence. One form terminated in cysteine-valine-leucine-serine (Ras-CVLS) the other in cysteine-valine-leucine-leucine (Ras-CVLL). Ras-CVLS is a substrate for the farnesyl protein transferase while Ras-CVLL is a substrate for geranylgeranyl protein transferase I. The cDNAs encoding these proteins were constructed so that the proteins contain an amino-terminal extension of 6 histidine residues.
 - 25 Both proteins were expressed in Escherichia coli and purified using metal chelate affinity chromatography. The radiolabelled isoprenyl pyrophosphate substrates, [³H]farnesyl pyrophosphate and [³H]geranylgeranyl pyrophosphate, were purchased from DuPont/New England Nuclear.

Several methods for measuring farnesyl protein transferase activity have been described (Reiss et al 1990, *Cell* **62**: 81; Schaber et al 1990, *J. Biol. Chem.* **265**: 14701; Manne et al 1990, *PNAS* **87**: 7541; and Barbacid & Manne 1993, U.S. Patent No. 5,185,248). The 5 activity was assayed by measuring the transfer of [³H]farnesyl from [³H]farnesyl pyrophosphate to Ras-CVLS using conditions similar to those described by Reiss et al. 1990 (*Cell* **62**: 81) The reaction mixture contained 40 mM Hepes, pH 7.5; 20 mM magnesium chloride; 5 mM dithiothreitol; 0.25 µM [³H]farnesyl pyrophosphate; 10 ml Q-Sepharose- 10 purified farnesyl protein transferase; the indicated concentration of tricyclic compound or dimethylsulfoxide (DMSO) vehicle control (5% DMSO final); and 5 mM Ras-CVLS in a total volume of 100 ml. The reaction was allowed to proceed for 30 minutes at room temperature and then stopped with 0.5 ml of 4% sodium dodecyl sulfate (SDS) followed 15 by 0.5 ml of cold 30% TCA. Samples were allowed to sit on ice for 45 minutes and precipitated Ras protein was then collected on GF/C filter paper mats using a Brandel cell harvester. Filter mats were washed once with 6% TCA, 2% SDS and radioactivity was measured in a Wallac 1204 Betaplate BS liquid scintillation counter. Percent inhibition was 20 calculated relative to the DMSO vehicle control.

2. Cell-Based Assay: Transient expression of val¹²-Ha-Ras-CVLS and val¹²-Ha-Ras-CVLL in COS monkey kidney cells: Effect of farnesyl protein transferase inhibitors on Ras processing and on disordered cell growth induced by transforming Ras.

25 COS monkey kidney cells were transfected by electroporation with the plasmid pSV-SPORT (Gibco/BRL) containing a cDNA insert encoding either Ras-CVLS or Ras-CVLL, leading to transient overexpression of a Ras substrate for either farnesyl protein transferase or geranylgeranyl protein transferase I, respectively (see above).

30 Following electroporation, cells were plated into 6-well tissue culture dishes containing 1.5 ml of Dulbecco's-modified Eagle's media (GIBCO, Inc.) supplemented with 10% fetal calf serum and the appropriate farnesyl protein transferase inhibitors. After 24 hours, media was removed and fresh media containing the appropriate drugs was re- 35 added.

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48 hours after electroporation cells were examined under the microscope to monitor disordered cell growth induced by transforming Ras. Cells expressing transforming Ras become more rounded and refractile and overgrow the monolayer, reminiscent of the transformed

5 phenotype. Cells were then photographed, washed twice with 1 ml of cold phosphate-buffered saline (PBS) and removed from the dish by scraping with a rubber policeman into 1 ml of a buffer containing 25 mM Tris, pH 8.0; 1 mM ethylenediamine tetraacetic acid; 1 mM phenylmethylsulfonyl fluoride; 50 mM leupeptin; and 0.1 mM pepstatin.

10 Cells were lysed by homogenization and cell debris was removed by centrifugation at 2000 x g for 10 min.

Cellular protein was precipitated by addition of ice-cold trichloroacetic acid and redissolved in 100 ml of SDS-electrophoresis sample buffer. Samples (5-10 ml) were loaded onto 14% polyacrylamide minigels (Novex, Inc.) and electrophoresed until the tracking dye neared the bottom of the gel. Proteins resolved on the gels were electroblotted onto nitrocellulose membranes for immunodetection.

Membranes were blocked by incubation overnight at 4°C in PBS containing 2.5% dried milk and 0.5% Tween-20 and then incubated with a Ras-specific monoclonal antibody, Y13-259 (Furth, M.E., et al., (1982), Monoclonal antibodies to the p21 products of the transforming gene of Harvey murine sarcome virus and of the cellular ras gene family, J. Virol. **43:** 294-304), in PBS containing 1% fetal calf serum for one hour at room temperature. After washing, membranes were incubated for one hour at room temperature with a 1:5000 dilution of secondary antibody, rabbit anti-rat IgG conjugated to horseradish peroxidase, in PBS containing 1% fetal calf serum. The presence of processed and unprocessed Ras-CVLS or Ras-CVLL was detected using a colorimetric peroxidase reagent (4-chloro-1-naphthol) as described by the manufacturer (Bio-Rad).

Compounds of the invention exhibited the following biological activity.

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Table 2. FPT INHIBITION

EXAMPLE	FPT IC ₅₀ (μM)	EXAMPLE	FPT IC ₅₀ (μM)
1	0.39	25	47% @ 330nM
2	0.004	26	0.54
3	1.1	27	0.13
4	0.38	28	43% @ 110 nM
5	0.056	29	0.032
6	0.0065	30	36% @ 110 nM
7	0.022	31	0.058
8	0.014	32	12 % @ 88 nM
9	0.006	33	38.7% @93.4nM
10	0.019	45	0.028
11	0.076	46	0.078
12	0.061	47	0.068
13	0.015	48	0.009
14	0.016	49	0.27
15	0.063	50	0.014
16	>0.1	51	0.019
23	0.62	52	0.044
17	~0.1	53	0.010
18	9 % @ 93 nM	54	0.015
19	21% @ 0.06 μg/μL	55	0.017
20	0 % @ 92 nM	56	0.007

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21	35% @ 0.06 µg/µL	57	0.011
22	41% @ 0.06 µg/µL	58	0.0091
24	20 % @ 0.06 µg/µL	59	0.0033

Table 3. ACTIVITY IN COS CELLS

EXAMPLE	INHIBITION OF RAS PROCESSING IC ₅₀ (µM)
2	0.013
6	0.035
10	0.500
13	0.200
14	0.250
18	0.540
58	0.300
59	0.015

5

For preparing pharmaceutical compositions from the compounds described by this invention, inert, pharmaceutically acceptable carriers can be either solid or liquid. Solid form preparations include powders, tablets, dispersible granules, capsules, cachets and suppositories. The 10 powders and tablets may be comprised of from about 5 to about 70 percent active ingredient. Suitable solid carriers are known in the art, e.g. magnesium carbonate, magnesium stearate, talc, sugar, lactose. Tablets, powders, cachets and capsules can be used as solid dosage forms suitable for oral administration.

15 For preparing suppositories, a low melting wax such as a mixture of fatty acid glycerides or cocoa butter is first melted, and the active ingredient is dispersed homogeneously therein as by stirring. The

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molten homogeneous mixture is then poured into convenient sized molds, allowed to cool and thereby solidify.

Liquid form preparations include solutions, suspensions and emulsions. As an example may be mentioned water or water-propylene glycol solutions for parenteral injection.

Liquid form preparations may also include solutions for intranasal administration.

Aerosol preparations suitable for inhalation may include solutions and solids in powder form, which may be in combination with a pharmaceutically acceptable carrier, such as an inert compressed gas.

Also included are solid form preparations which are intended to be converted, shortly before use, to liquid form preparations for either oral or parenteral administration. Such liquid forms include solutions, suspensions and emulsions.

The compounds of the invention may also be deliverable transdermally. The transdermal compositions can take the form of creams, lotions, aerosols and/or emulsions and can be included in a transdermal patch of the matrix or reservoir type as are conventional in the art for this purpose.

Preferably the compound is administered orally.

Preferably, the pharmaceutical preparation is in unit dosage form. In such form, the preparation is subdivided into unit doses containing appropriate quantities of the active component, e.g., an effective amount to achieve the desired purpose.

The quantity of active compound in a unit dose of preparation may be varied or adjusted from about 0.1 mg to 1000 mg, more preferably from about 1 mg. to 300 mg, according to the particular application.

The actual dosage employed may be varied depending upon the requirements of the patient and the severity of the condition being treated. Determination of the proper dosage for a particular situation is within the skill of the art. Generally, treatment is initiated with smaller dosages which are less than the optimum dose of the compound. Thereafter, the dosage is increased by small increments until the optimum effect under the circumstances is reached. For convenience,

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the total daily dosage may be divided and administered in portions during the day if desired.

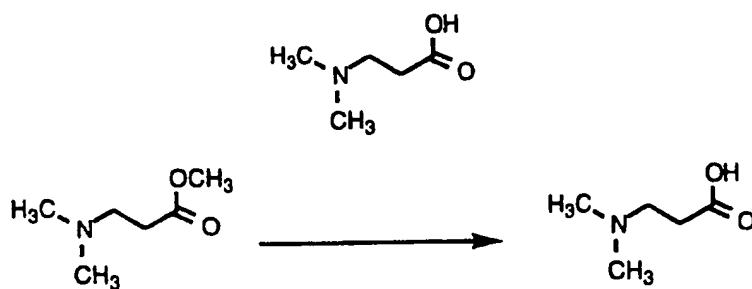
The amount and frequency of administration of the compounds of the invention and the pharmaceutically acceptable salts thereof will be
 5 regulated according to the judgment of the attending clinician considering such factors as age, condition and size of the patient as well as severity of the symptoms being treated. A typical recommended dosage regimen is oral administration of from 10 mg to 2000 mg/day preferably 10 to 1000 mg/day, in two to four divided doses to block tumor
 10 growth. The compounds are non-toxic when administered within this dosage range.

While the present invention has been described in conjunction with the specific embodiments set forth herein, many alternatives, modifications and variations thereof will be apparent to those of ordinary
 15 skill in the art. All such alternatives, modifications and variations are intended to fall within the spirit and scope of the present invention.

Compounds of the invention may be prepared according to the procedures described in WO 95/10516 published April 20, 1995, copending Application Serial No. 08/410,187 filed March 24, 1995,
 20 copending Application Serial No. 08/577,951 filed December 22, 1995, and copending Application Serial No. 08/615,760 filed March 13, 1996; the disclosures of each being incorporated herein by reference thereto; and according to the procedures described below.

25

PREPARATIVE EXAMPLE 1

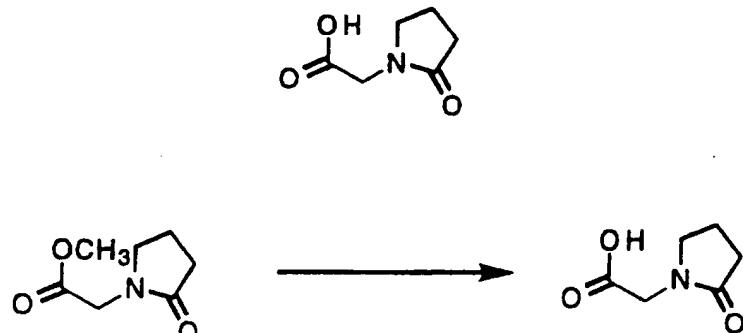


2 g (15 mmol) of methyl 3-(dimethyl amino) propionate was dissolved in 20 mL of EtOH and then 20 mL of 1M LiOH was added. The reaction mixture was stirred at room temperature for 16 hours. The
 30 solvents were stripped off. The resulting material in water was dissolved

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and pH was adjusted to ~ 6. The reaction mixture was concentrated to give the product. Mass Spec.: $MH^+ = 118$.

PREPARATIVE EXAMPLE 2

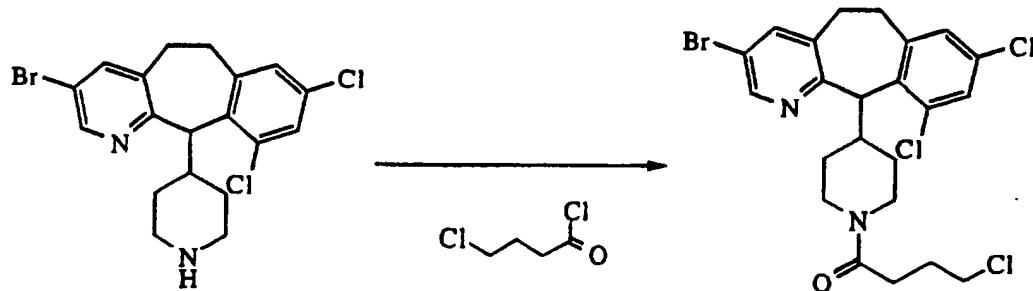


5 2 g (12.7 mmol) of methyl 2-oxo-1-pyrrolidine acetate was dissolved in 20 mL of EtOH and then 20 mL of 1M LiOH was added. The reaction mixture was stirred at room temperature for 16 hours. The solvents were stripped off. The resulting material was dissolved in water and the pH was adjusted to ~ 4. The reaction mixture was concentrated
10 to give the product. Mass Spec.: $MH^+ = 144$.

PREPARATIVE EXAMPLE 3

(+)-4-(3-BROMO-8,10-DICHLORO-6,11-DIHYDRO-5H-BENZO[5,6]-CYCLO-HEPTA[1,2-b]PYRIDIN-11-YL)-1-[4-(CHLORO)-1-OXOBUTYL]

15 PIPRIDINE

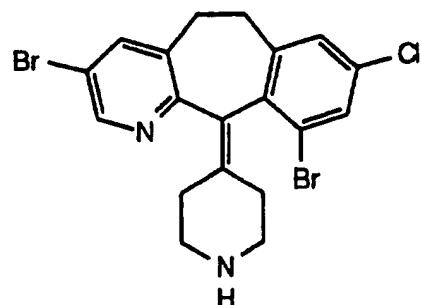


4-bromobutyric acid (5g, 29.9 mmol) was dissolved in 50 mL of CH_2Cl_2 and thionyl chloride (35.6 g, 299 mmol) was then added. The reaction mixture was stirred at room temperature for ~ 16h. Excess
20 thionyl chloride was removed by rotary evaporation and final traces were chased off with toluene. The crude product was dried under high vacuum to obtain 4.47g of crude acid chloride. To this acid chloride (0.65g , 3.5 mmol) was added the title compound of preparative Example

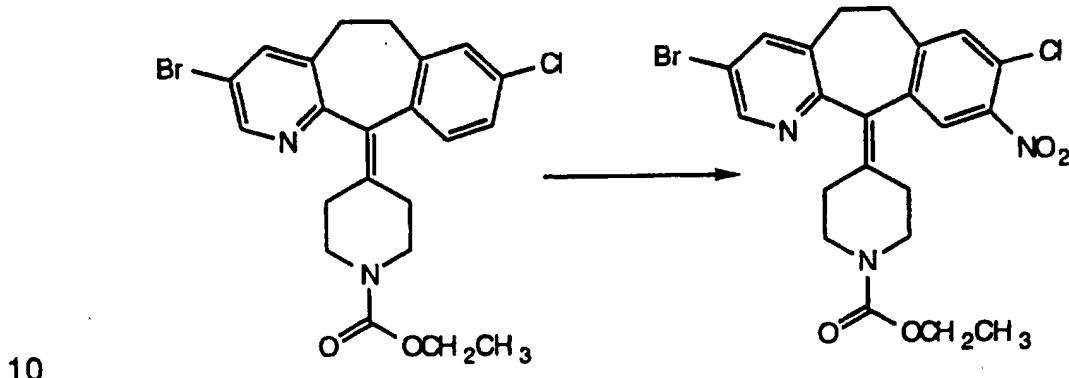
-34-

7, (1.0g, 2.3 mmol), and triethylamine(0.7 mL, 5.2 mmol) and then dissolved in 10 mL of CH₂Cl₂. Reaction mixture was stirred at room temperature for 16h. It was extracted with sat. NaHCO₃ and the CH₂Cl₂ fraction was dried over MgSO₄ and concentrated to give 0.63 g of the
 5 title compound FAB-MS: MH⁺ = 531

PREPARATIVE EXAMPLE 4



Step A:



10

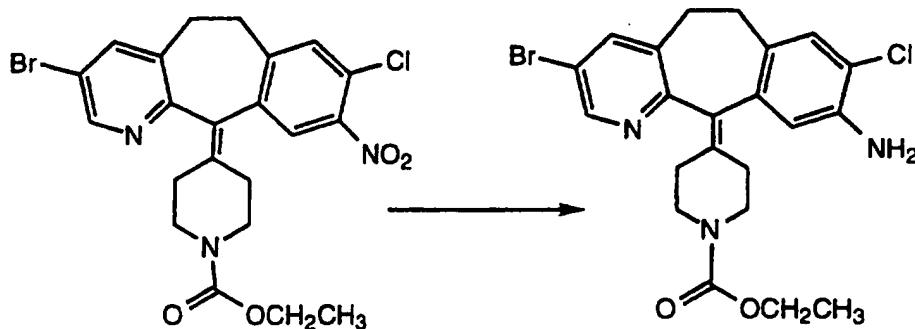
15 g (38.5 mmol) of 4-(8-chloro-3-bromo-5,6-dihydro-11H-benzo[5,6]cyclohepta[1,2-b]pyridin-11-ylidene)-1-piperidine-1-carboxylic acid ethyl ester and 150 mL of concentrated H₂SO₄ at -5°C, then add 3.89 g (38.5 mmol) of KNO₃ were combined and stirred for 4 hours. The mixture was poured into 3 L of ice and basified with 50% NaOH (aqueous). The mixture was extracted with CH₂Cl₂, dried over MgSO₄, then filtered and concentrated *in vacuo* to a residue. The residue was recrystallized from acetone to give 6.69 g of the product. ¹H NMR (CDCl₃, 200 MHz): 8.5 (s, 1H); 7.75 (s, 1H); 7.6 (s, 1H); 7.35 (s, 1H); 4.15 (q, 2H); 3.8 (m, 2H); 3.5-3.1 (m, 4H); 3.0-2.8 (m, 2H); 2.6-2.2 (m, 4H); 1.25 (t, 3H).

15

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-35-

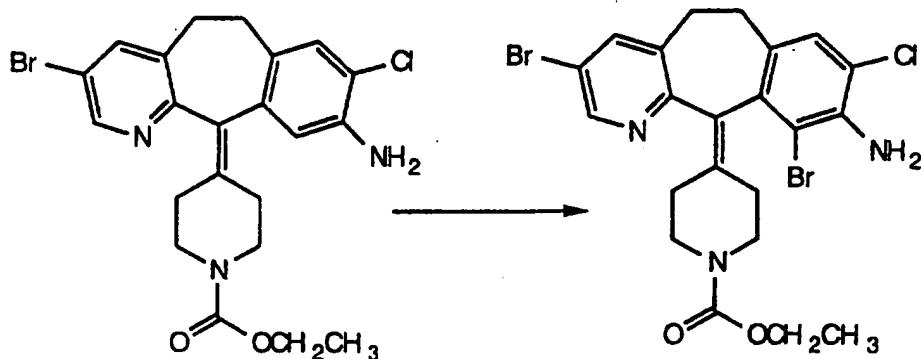
Step B:



5 6.69 g (13.1 mmol) of the product of Step A and 100 mL of 85% EtOH/water were combined, then 0.66 g (5.9 mmol) of CaCl_2 and 6.56 g (117.9 mmol) of Fe were added and the mixture was heated at reflux overnight. The hot reaction mixture was filtered through celite® and the filter cake was rinsed with hot EtOH. The filtrate was concentrated *in vacuo* to give 7.72 g of the product. Mass Spec.: $\text{MH}^+ = 478.0$

10

Step C:



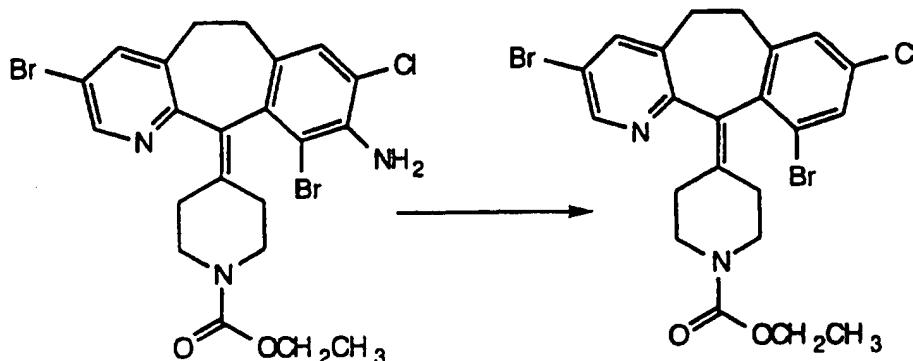
7.70 g of the product of Step B and 35 mL of HOAc, were combined then 45 mL of a solution of Br_2 in HOAc was added and the 15 mixture was stirred at room temperature overnight. 300 mL of 1 N NaOH (aqueous) was added, then 75 mL of 50% NaOH (aqueous) was added and the mixture was extracted with EtOAc. The extract was dried over MgSO_4 and concentrated *in vacuo* to a residue. The residue was chromatographed (silica gel, 20%-30% EtOAc/hexane) to give 3.47 g of 20 the product (along with another 1.28 g of partially purified product). Mass Spec.: $\text{MH}^+ = 555.9$.

$^1\text{H NMR}$ (CDCl_3 , 300 MHz): 8.5 (s, 1H); 7.5 (s, 1H); 7.15 (s, 1H); 4.5 (s,

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2H); 4.15 (m, 3H); 3.8 (br s, 2H); 3.4-3.1 (m, 4H); 9-2.75 (m, 1H); 2.7-2.5 (m, 2H); 2.4-2.2 (m, 2H); 1.25 (m, 3H).

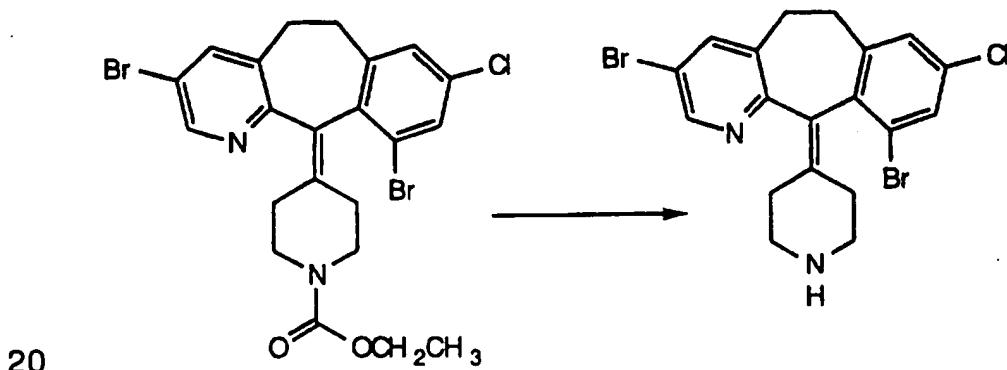
Step D:



5 0.557 g (5.4 mmol) of t-butylnitrite and 3 mL of DMF were combined, and the mixture was heated at 60°-70°C. A mixture of 2.00 g (3.6 mmol) of the product of Step C and 4 mL of DMF, was slowly added dropwise and then the mixture was cooled to room temperature.
 10 Another 0.64 mL of t-butylnitrite was added at 40°C and the mixture was reheated to 60°-70°C for 0.5 hrs. The mixture was cooled to room temperature and poured into 150 mL of water. The mixture was extracted with CH₂Cl₂, the extract was dried over MgSO₄ and concentrated *in vacuo* to a residue. The residue was chromatographed (silica gel, 10%-20% EtOAc/hexane) to give 0.74 g of the product. Mass Spec.: MH⁺ = 541.0.

15 ¹H NMR (CDCl₃, 200 MHz): 8.52 (s, 1H); 7.5 (d, 2H); 7.2 (s, 1H); 4.15 (q, 2H); 3.9-3.7 (m, 2H); 3.5-3.1 (m, 4H); 3.0-2.5 (m, 2H); 2.4-2.2 (m, 2H); 2.1-1.9 (m, 2H); 1.26 (t, 3H).

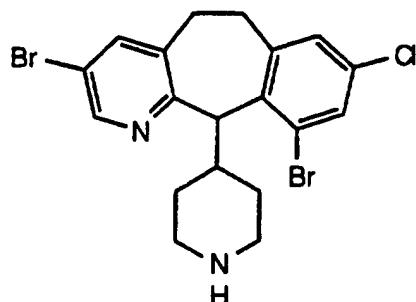
Step E:



-37-

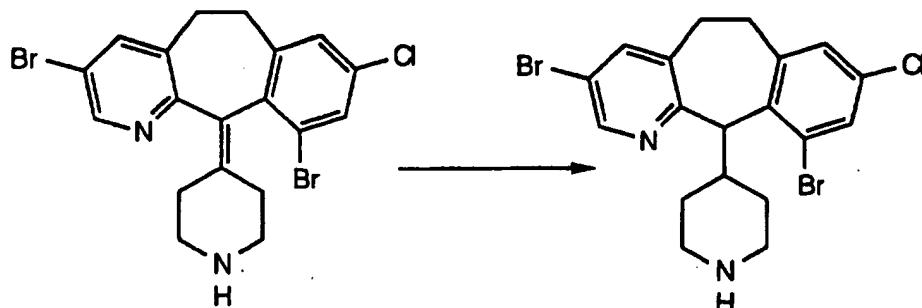
0.70 g (1.4 mmol) of the product of Step D and 8 mL of concentrated HCl (aqueous) were combined and the mixture was heated at reflux overnight. 30 mL of 1 N NaOH (aqueous) was added, then 5 mL of 50% NaOH (aqueous) and the mixture was extracted with CH₂Cl₂. The extract was dried over MgSO₄ and concentrated *in vacuo* to give 0.59 g of the title compound. Mass Spec.: M⁺ = 468.7. m.p. = 123.9°-124.2°C.

PREPARATIVE EXAMPLE 5



10 [racemic as well as (+)- and (-)-isomers]

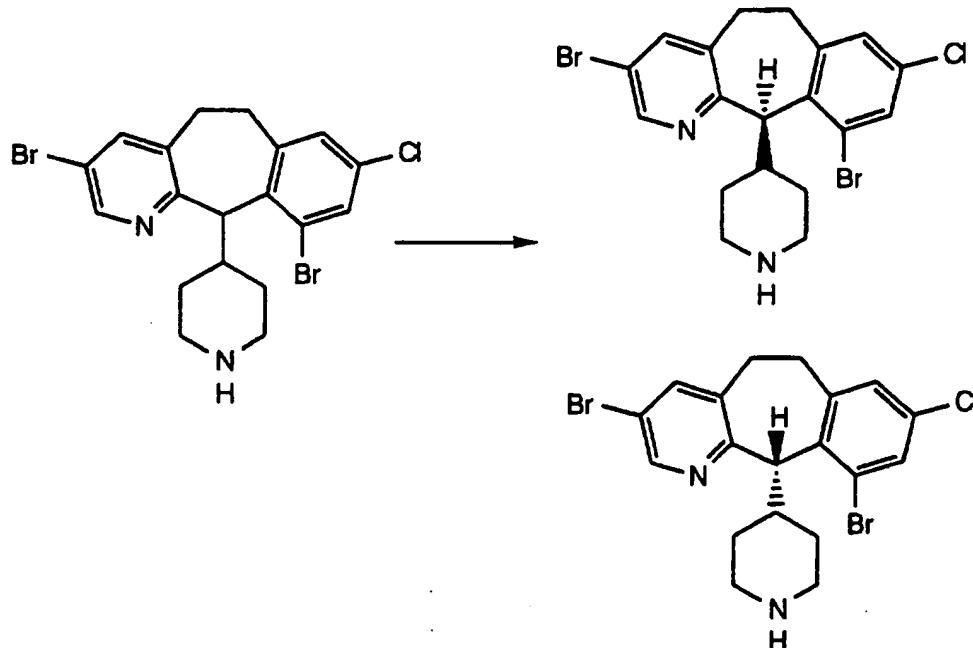
Step A:



A solution of 8.1 g of the title compound from Preparative Example 4 in toluene was prepared and 17.3 mL of a 1M solution of DIBAL in toluene was added. The mixture was heated at reflux and another 21 mL of 1 M DIBAL/toluene solution was slowly added dropwise over a period of 40 min. The reaction mixture was cooled to about 0°C and 700 mL of 1 M HCl (aqueous) was added. The organic phase was separated and discarded. The aqueous phase was washed with CH₂Cl₂, the extract was discarded, then the aqueous phase was basified by adding 50% NaOH (aqueous). The mixture was extracted with CH₂Cl₂, the extract was dried over MgSO₄ and concentrated *in vacuo* to give 7.30 g of the title compound, which is a racemic mixture of enantiomers.

-38-

Step B - Separation of Enantiomers:

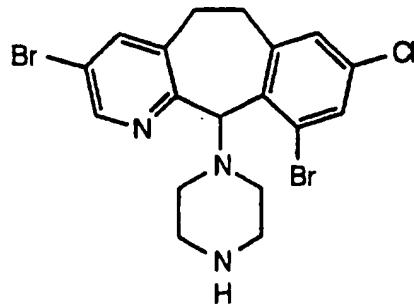


The racemic title compound of Step A was separated by preparative chiral chromatography (Chiralpack AD, 5 cm X 50 cm column, using 20% iPrOH/hexane + 0.2% diethylamine), to give the (+)-isomer and the (-)-isomer of the title compound.

Physical chemical data for (+)-isomer: m.p. = 148.8°C;
 Mass Spec. $\text{MH}^+ = 472$; $[\alpha]_D^{25} = +65.6^\circ$ (12.93 mg/ 2mL MeOH).

Physical chemical data for (-)-isomer: m.p. = 112°C;
 Mass Spec. $\text{MH}^+ = 472$; $[\alpha]_D^{25} = -65.2^\circ$ (3.65 mg/ 2mL MeOH).

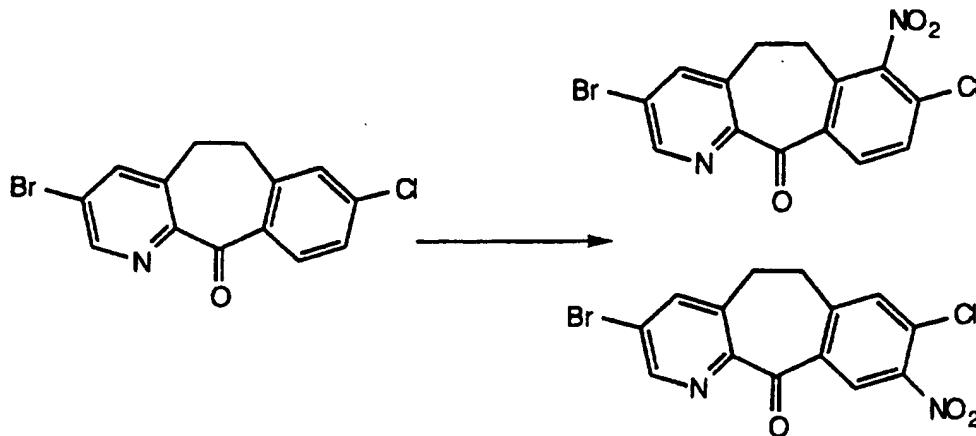
PREPARATIVE EXAMPLE 6



[racemic as well as (+)- and (-)-isomers]

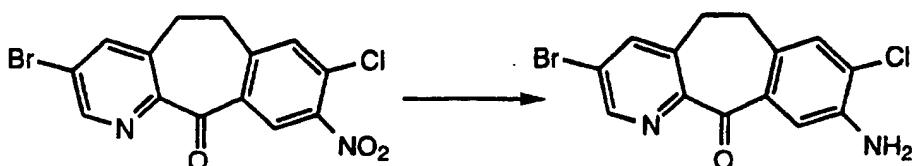
-39-

Step A:



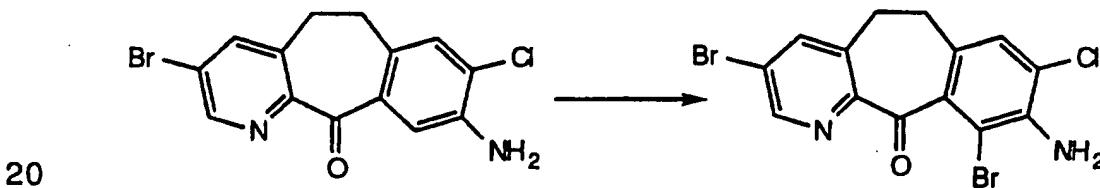
40.0 g (0.124 mole) of the starting ketone and 200 mL of H₂SO₄
5 were combined and cooled to 0°C. 13.78 g (0.136 mole) of KNO₃ were
slowly added over a period of 1.5 hrs., then the mixture was warmed to
room temperature and stirred overnight. The reaction was worked up
using substantially the same procedure as described for Preparative
Example 4, Step A. Chromatography (silica gel, 20%, 30%, 40%, 50%
10 EtOAc/hexane, then 100% EtOAc) gave 28 g of the 9-nitro product, along
with a smaller quantity of the 7-nitro product and 19 g of a mixture of the
7-nitro and 9-nitro compounds.

Step B:



15 28 g (76.2 mmol) of the 9-nitro product of Step A, 400 mL of 85% EtOH/water, 3.8 g (34.3 mmol) of CaCl_2 and 38.28 g (0.685 mole) of Fe were reacted using substantially the same procedure as described for Preparative Example 4, Step C, to give 24 g of the product.

Step C:

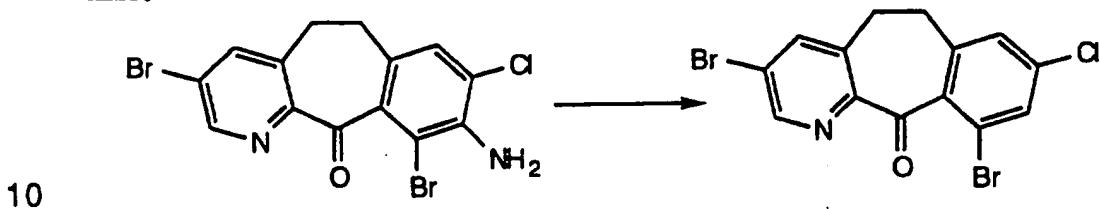


-40-

13 g (38.5 mmol) of the product of Step B and 140 mL of HOAc were combined and slowly added to a solution of 2.95 mL (57.8 mmol) of Br₂ in 10 mL of HOAc over a period of 20 min. The reaction mixture was stirred at room temperature, then concentrated *in vacuo* to a residue.

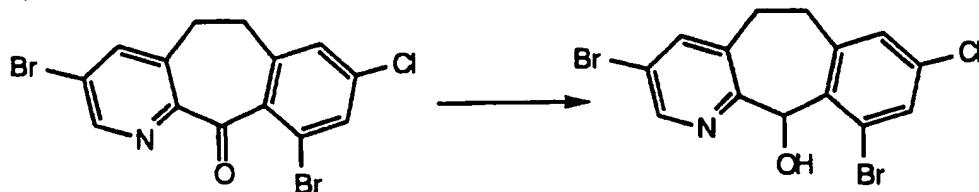
5 CH₂Cl₂ and water were added, then the pH was adjusted to 8-9 with 50% NaOH (aqueous). The organic phase was washed with water, then brine and dried over Na₂SO₄ and concentrated *in vacuo* to give 11.3 g of the product.

Step D:



10 100 mL of concentrated HCl (aqueous) was cooled to 0°C, then 5.61 g (81.4 mmol) of NaNO₂ were added and the mixture was stirred for 10 min. 11.3 g (27.1 mmol) of the product of Step C was slowly added in portions and the mixture was stirred at 0°-3°C for 2.25 hrs. 180 mL of 15 50% H₃PO₂ (aqueous) was slowly added in portions and the mixture was allowed to stand at 0°C overnight. 150 mL of 50% NaOH was slowly added in portions over 30 min., and the pH was adjusted to 9. The mixture was then extracted with CH₂Cl₂. The extract was washed with water, then brine and dried over Na₂SO₄. The mixture was 20 concentrated *in vacuo* to a residue and chromatographed (silica gel, 2% EtOAc/CH₂Cl₂) to give 8.6 g of the product.

Step E:

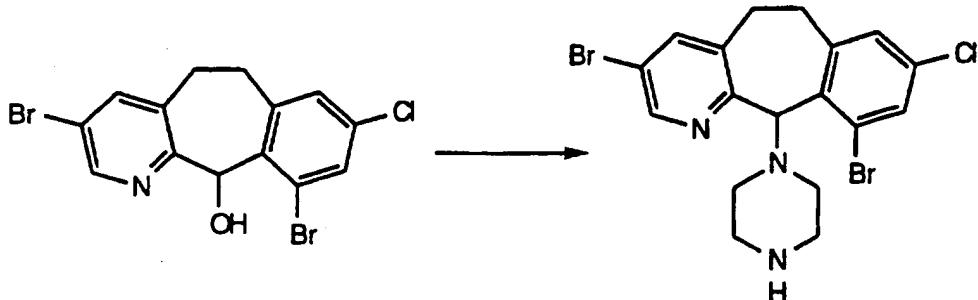


25 8.6 g (21.4 mmol) of the product of Step D and 300 mL of MeOH were combined and cooled to 0°-2°C. 1.21 g (32.1 mmol) of NaBH₄ was added and the mixture was stirred at -0°C for 1 hr. Another 0.121 g (3.21 mmol) of NaBH₄, was added and the mixture was stirred for 2 hr. at 0°C, then allowed to stand overnight at 0°C. It was concentrated *in*

-41-

vacuo to a residue then the residue was partitioned between CH₂Cl₂ and water. The organic phase was separated and concentrated *in vacuo* (50°C) to give 8.2 g of the product.

Step F:

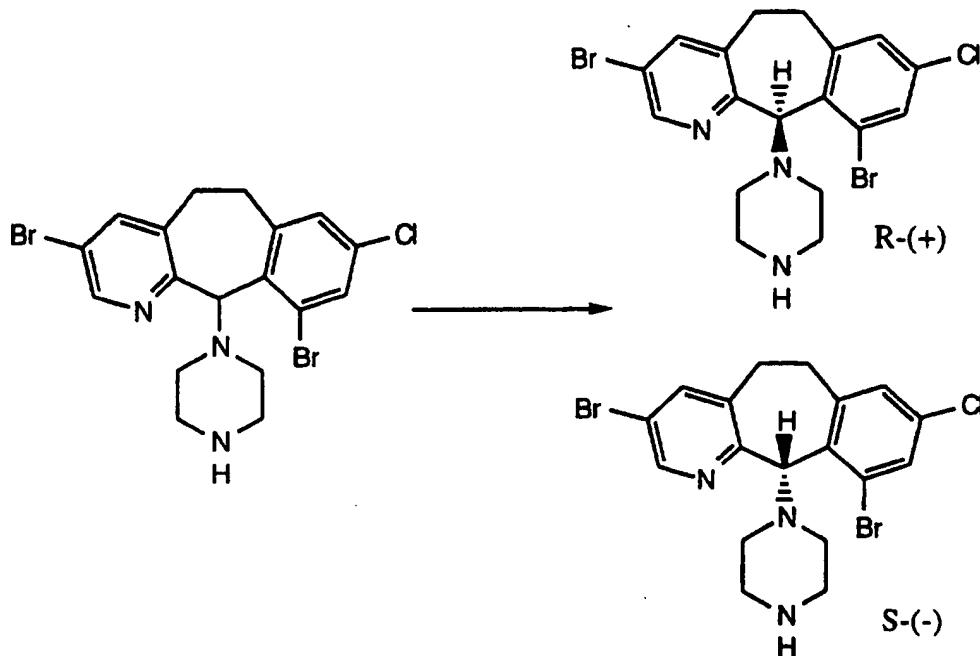


5

8.2 g (20.3 mmol) of the product of Step E was combined with 160 mL of CH₂Cl₂, cooled to 0°C, then 14.8 mL (203 mmol) of SOCl₂ was slowly added dropwise over a 30 minute period. The mixture was warmed to room temperature and stirred for 4.5 hrs., then concentrated *in vacuo* to a residue. CH₂Cl₂ was added and the mixture was washed with 1 N NaOH (aqueous) then brine and dried over Na₂SO₄. The residue was concentrated *in vacuo* to a residue, then dry THF was added and 8.7 g (101 mmol) of piperazine was added and the mixture was stirred at room temperature overnight. The residue was concentrated *in vacuo* to a residue, CH₂Cl₂, was added and the mixture was washed with 0.25 N NaOH (aqueous), water, then brine dried over Na₂SO₄ and concentrated *in vacuo* to give 9.46 g of the crude product. Chromatography (silica gel, 5% MeOH/CH₂Cl₂ + NH₃) gave 3.59 g of the title compound, as a racemate. ¹H NMR (CDCl₃, 200 MHz): 8.43 (d, 1H); 7.55 (d, 1H); 7.45 (d, 1H); 7.11 (d, 1H); 5.31 (s, 1H); 4.86-4.65 (m, 1H); 3.57-3.40 (m, 1H); 2.98-2.55 (m, 6H); 2.45-2.20 (m, 5H).

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Step G - Separation of Enantiomers:

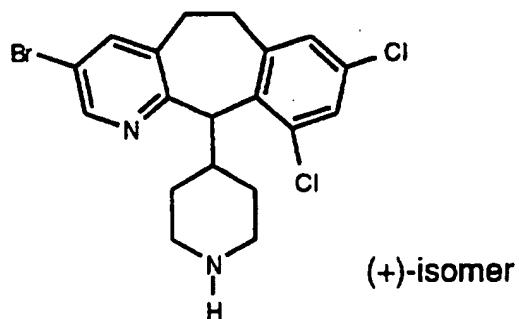


The racemic title compound from Step F (5.7 g) was chromatographed as described for Preparative Example 6, Step D,
 5 using 30% iPrOH/hexane + 0.2% diethylamine, to give 2.88 g of the R-(+)-isomer and 2.77 g of the S-(-)-isomer of the title compound.

Physical chemical data for the R-(+)-isomer: Mass Spec. MH^+ = 472.0; $[\alpha]_D^{25} = +12.1^\circ$ (10.9 mg/ 2mL MeOH).

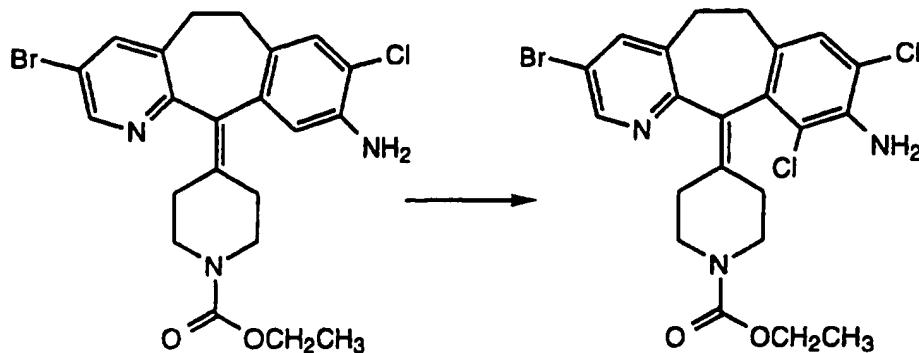
Physical chemical data for the S-(-)-isomer: Mass Spec. MH^+ = 10 472.0; $[\alpha]_D^{25} = -13.2^\circ$ (11.51 mg/ 2mL MeOH).

Preparative Example 7



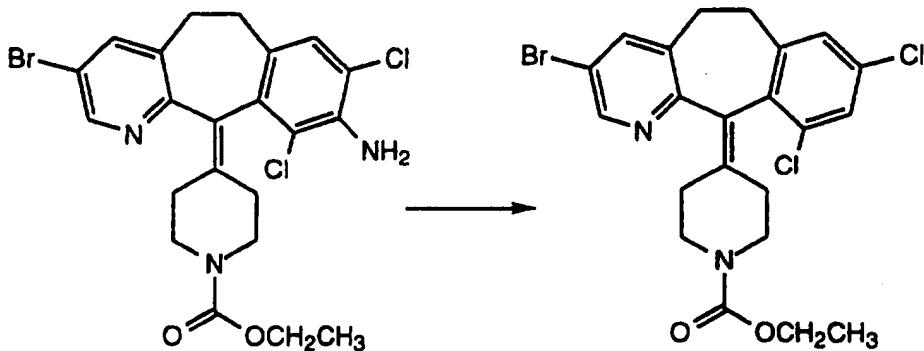
-43-

Step A:



Dissolve 9.90 g (18.9 mmol) of the product of Preparative Example 4, Step B, in 150 mL CH_2Cl_2 and 200 mL of CH_3CN and heat to 60°C. Add 2.77 g (20.8 mmol) N-chlorosuccinimide and heat to reflux for 3 h., monitoring the reaction by TCL (30% $\text{EtOAc}/\text{H}_2\text{O}$). Add an additional 2.35 g (10.4 mmol) of N-chlorosuccinimide and reflux an additional 45 min. Cool the reaction mixture to room temperature and extract with 1N NaOH and CH_2Cl_2 . Dry the CH_2Cl_2 layer over MgSO_4 , filter and purify by flash chromatography (1200 mL normal phase silica gel, eluting with 30% $\text{EtOAc}/\text{H}_2\text{O}$) to obtain 6.24 g of the desired product. M.p. 193-195.4°C.

Step B:

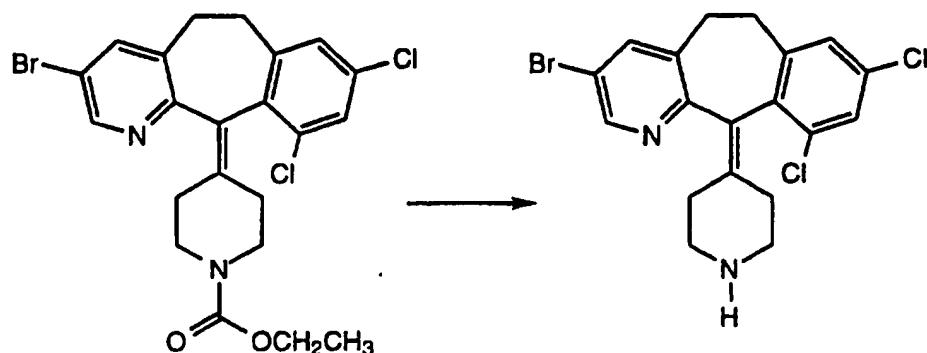


15 To 160 mL of conc. HCl at -10°C add 2.07 g (30.1 mmol) NaNO₂ and stir for 10 min. Add 5.18 g (10.1 mmol) of the product of Step A and warm the reaction mixture from -10°C to 0°C for 2 h. Cool the reaction to -10°C, add 100 mL H₃PO₂ and let stand overnight. To extract the reaction mixture, pour over crushed ice and basify with 50% NaOH/CH₂Cl₂. Dry the organic layer over MgSO₄, filter and concentrate to dryness. Purify by flash chromatography (600 mL normal phase silica

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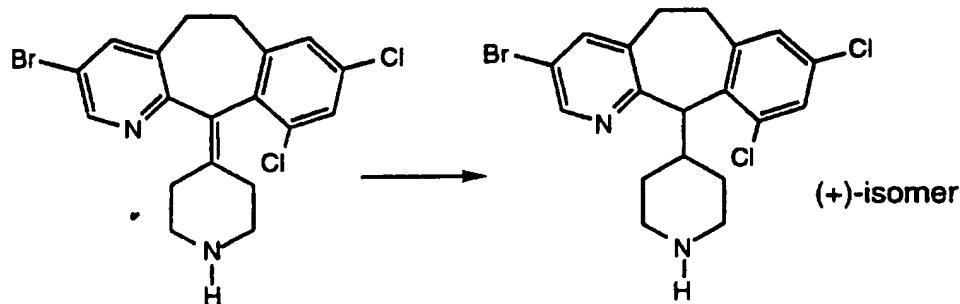
gel, eluting with 20% EtOAc/hexane) to obtain 3.98 g of product. Mass spec.: $MH^+=497.2$.

Step C:



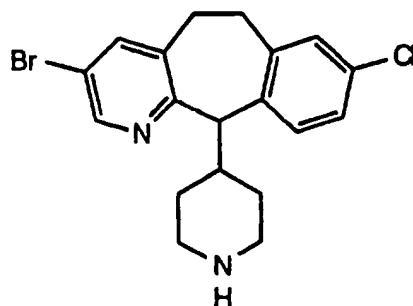
5 Dissolve 3.9 g of the product of Step B in 100 mL conc. HCl and reflux overnight. Cool the mixture, basify with 50 % w/w NaOH and extract the resultant mixture with CH_2Cl_2 . Dry the CH_2Cl_2 layer over $MgSO_4$, evaporate the solvent and dry under vacuum to obtain 3.09 g of the desired product. Mass spec.: $MH^+=424.9$.

10 Step D:



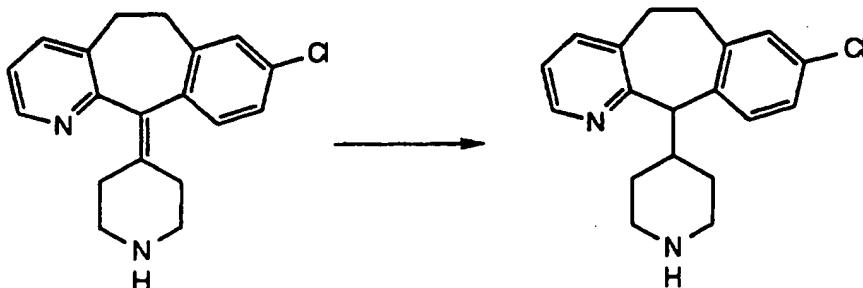
Using a procedure similar to that described in Preparative Example 6, obtain 1.73 g of the desired product, m.p. 169.6-170.1°C; $[a]_D^{25} = +48.2^\circ$ (c=1, MeOH).

15 Preparative Example 8



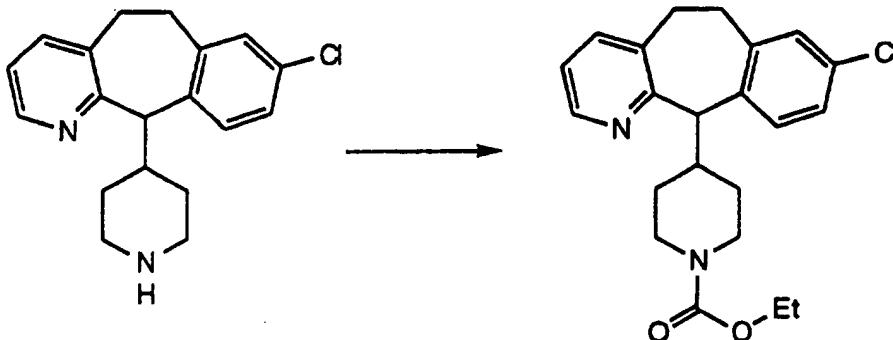
-45-

Step A:



Combine 82.0 g (0.26 mole) of the product of Preparative Example 1, Step G, of WO 95/10516, and 1 L of toluene, then add 20.06 g (0.53 mole) of LiAlH₄ and heat the reaction mixture at reflux overnight. Cool the mixture to room temperature and add ~1 L of Et₂O, followed by dropwise addition of saturated Na₂SO₄ (aqueous) until a precipitate forms. Filter and stir the filtrate over MgSO₄ for 30 minutes, then concentrate *in vacuo* to give the product compound in 83% yield. Mass Spec.: MH⁺ = 313

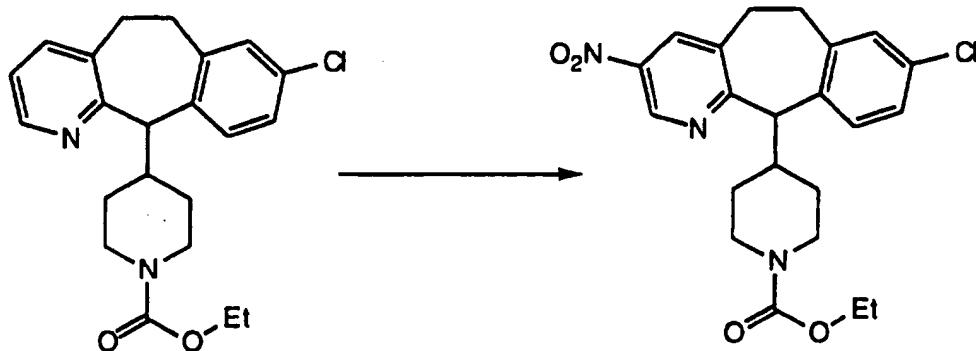
Step B:



Combine 24.32 g (74.9 mmol) of the Product from Step A, 500 mL of toluene, 83 mL of Et₃N and 65.9 mL of ethyl chloroformate and heat the mixture at reflux overnight. Cool to 25°C, pour into 200 mL of water and extract with EtOAc. Dry the extract over MgSO₄, concentrate *in vacuo* to a residue and chromatograph (silica gel, 50% EtOAc/hexane) to give 15 g of the product compound. Mass Spec.: MH⁺ = 385.

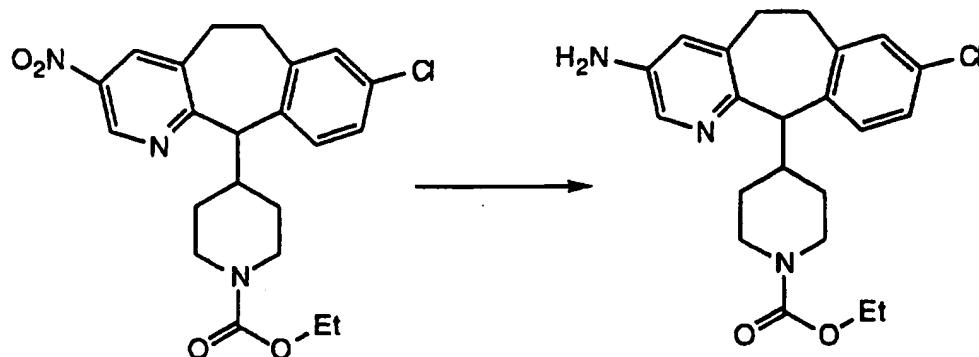
-46-

Step C:



Dissolve 3.2 g (10.51 mmol) of tetra-n-butylammonium nitrate in 25 mL of CH_2Cl_2 and add 2.2 g (10.51 mmol, 1.5 mL) of TFAA. Cool to 5 0°C and add the mixture (via cannula) to a solution of 3.68 g (9.56 mmol) of the product of Step B in 50 mL of CH_2Cl_2 at 0°C , then stir at 0°C for 3 hours. Allow the mixture to warm to 25°C while stirring overnight, then extract with saturated NaHCO_3 (aqueous) and dry over MgSO_4 . Concentrate *in vacuo* to a residue and chromatograph (silica gel, 30% 10 EtOAc/hexane) to give 1.2 g of the product compound. Mass Spec.: $\text{MH}^+ = 430$

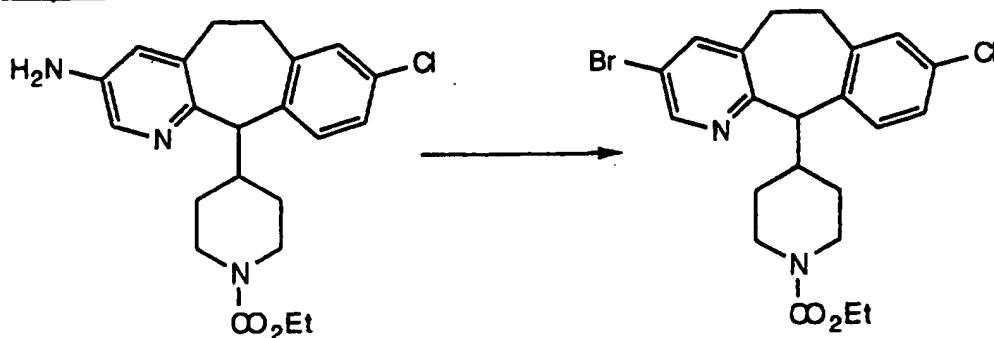
Step D:



Combine 2.0 g (4.7 mmol) of the Product of Step C and 150 mL of 15 85% EtOH (aqueous), add 2.4 g (42 mmol) of Fe filings and 0.24 g (2.1 mmol) of CaCl_2 , and heat at reflux for 16 hours. Filter the hot mixture through a bed of celite®, wash the celite® with hot EtOH. Concentrate the filtrate *in vacuo* to give a 100% yield of the product compound. Mass Spec.: $\text{MH}^+ = 400$.

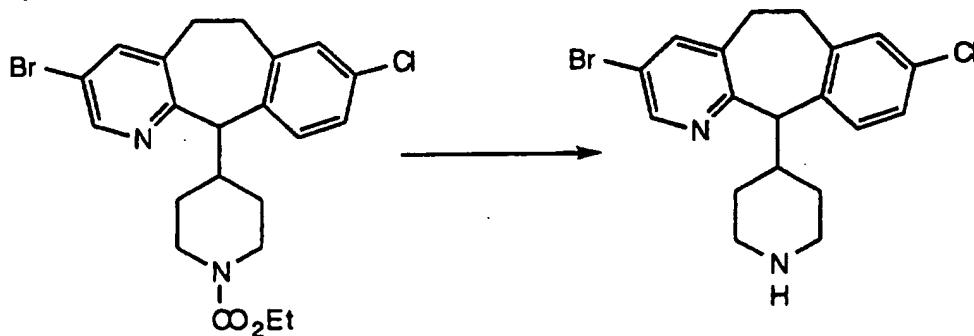
-47-

Step E:



Combine 2.0 g (5.2 mmol) of the Product of Step D and 20 mL of 48% HBr, cool the mixture to -5°C. Add 1.4 mL of bromine and stir the
 5 mixture at -5°C for 15 minutes and slowly add a solution of 1.07 g (15.5 mmol) of NaNO₂ in 10 mL of water. Stir for 45 minutes, then quench with 50% NaOH (aqueous) to pH ~10. Extract with EtOAc, dry the combined extracts over MgSO₄ and concentrate *in vacuo* to give the product compound. Mass Spec.: MH⁺ = 465

10 Step F:



Hydrolyze 4.0 g of the Product of Step E via substantially the same process as described for Example 358, Step A, of WO 95/10516, to give 1.39 g of the product compound. Mass Spec.: MH⁺ = 392

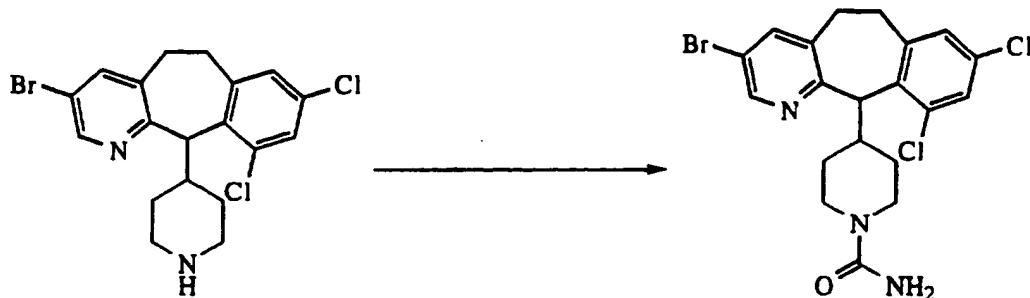
15

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EXAMPLE 1

(+)-4-(3-BROMO-8,10-DICHLORO-6,11-DIHYDRO-5H-BENZO[5,6]-CYCLOHEPTA[1,2-b]PYRIDIN-11-YL)-1-PIPERIDINE CARBOXAMIDE

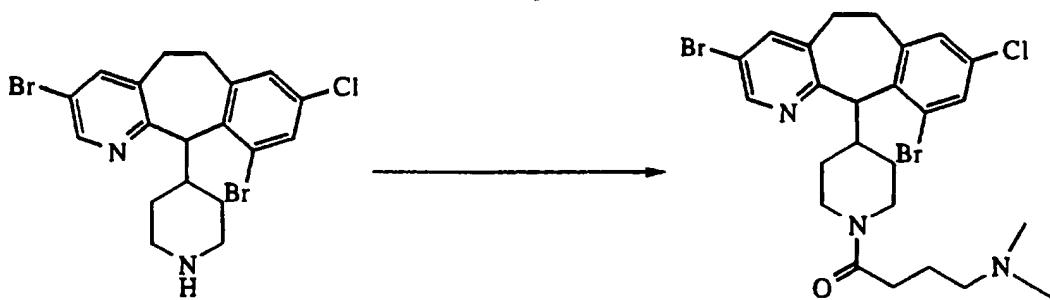


5 The title compound from preparative example 7 Step D (0.3 g, 0.7 mmol) and trimethylsilyl isocyanate (1.6 g, 2 mL, 14.1 mmol) were dissolved in CH₂Cl₂ (6 mL) and reaction stirred under nitrogen at room temperature for 72 h. Saturated aqueous sodium bicarbonate (20 mL) was then added. The desired product was extracted with CH₂Cl₂.

10 Combined CH₂Cl₂ extracts were dried over MgSO₄ and concentrated in vacuo to give the title compound as a white solid (0.3 g, 97% yield, mp = 101.9-102.8 °C, MH⁺ = 470).

EXAMPLE 2

15 (+)-4-(3,10-DIBROMO-8-CHLORO-6,11-DIHYDRO-5H-BENZO[5,6]-CYCLO-HEPTA[1,2-b]PYRIDIN-11-YL)-1-[4-(DIMETHYLAmino)-1-OXOBUTYL] PIPERIDINE



The title compound from Example 5 Step B -(+isomer), was dissolved in DMF (7mL) and then cooled to ~ 4 °C. 4-(Dimethylamino)-butyric acid hydrochloride salt, (0.13 g, 0.83 mmol) was then added followed by DEC (0.16 g, 0.83 mmol), HOBT (0.11 g, 0.83 mmol), and 4-methylmorpholine (0.08 g, 91 mM, 0.83 mmol) the reaction was then stirred at room temperature overnight. The reaction mixture was concentrated in vacuo to a residue that was partitioned between CH₂Cl₂ and Sat.

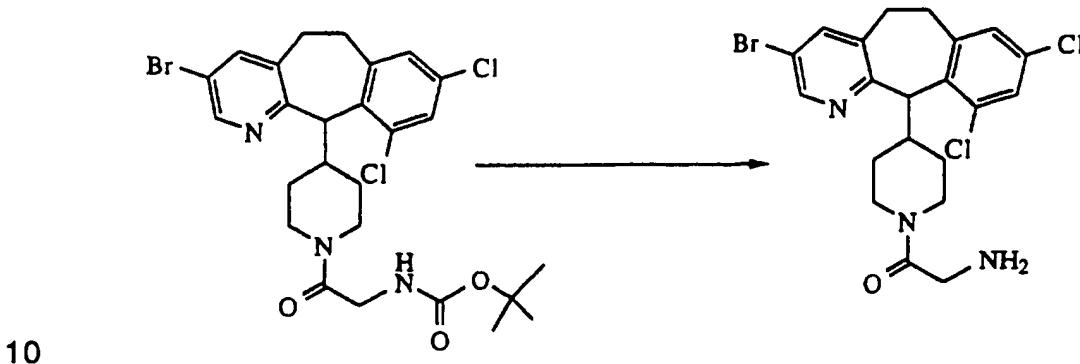
-49-

NaHCO₃ (aqueous). The aqueous phase was extracted further with CH₂Cl₂. Combined CH₂Cl₂ fractions were dried over MgSO₄ and concentrated in vacuo to give a residue that was chromatographed on silica gel column using 10 % (ammonia saturated methanol)/ CH₂Cl₂ eluent to give the title compound as a white solid (0.3 g, 81% yield, mp = 78-80 °C, MH⁺ = 584).

5 eluent to give the title compound as a white solid (0.3 g, 81% yield, mp = 78-80 °C, MH⁺ = 584).

EXAMPLE 3

(+)-1-(AMINOACETYL)-4-(3-BROMO-8,10-DICHLORO-6,11-DIHYDRO-5H-BENZO[5,6]CYCLO-HEPTA[1,2-b]PYRIDIN-11-YL) PIPERIDINE



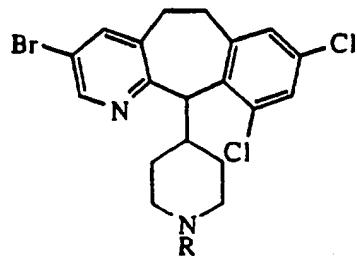
The title compound of Example 26 (0.6 g, 1.02 mmol) was dissolved in CH₂Cl₂ (6 mL) and trifluoroacetic acid (6 mL) was then added. The reaction mixture was stirred at room temperature for 4h. It was then poured into ice and the pH was adjusted to 10 using 50%(w/v) aqueous NaOH. Reaction mixture was extracted with CH₂Cl₂. Combined CH₂Cl₂ extracts were washed with H₂O, brine, and dried over Na₂SO₄. The solvents were removed rotary evaporation to give the title compound as a white solid ((0.447 g, mp = 81-122 °C, MH⁺ = 484)

15 combined CH₂Cl₂ extracts were washed with H₂O, brine, and dried over Na₂SO₄. The solvents were removed rotary evaporation to give the title compound as a white solid ((0.447 g, mp = 81-122 °C, MH⁺ = 484)

By essentially the same procedure as set forth in Example 2, but 20 using the carboxylic acids in column 1 of Table 1 below, in place of 4-(Dimethylamino)butyric acid hydrochloride salt, and using (+)-4-(3-bromo-8,10-dichloro-6,11-dihydro-5H-benzo[5,6]cyclo-hepta[1,2-b]pyridin-11-yl)-1-piperidine instead of (+)-4-(3,10-dibromo-8-chloro-6,11-dihydro-5H-benzo[5,6]cyclo-hepta[1,2-b]pyridin-11-yl)-1-piperidine, 25 (Title compound from Preparative example 7, Step D), one can obtain final products listed in column 2 of Table 1.

The R group in Table 1 refers to compounds of formula (1.0)", just below.

-50-



(1.0)''

Table 1

EXS	CARBOXYLIC ACID	-R	FINAL PRODUCTS
4			Solid mp 80-81 °C MS MH+ = 512
5			Solid mp 84-85 °C MS MH+ = 526
6			Solid mp 78-79 °C MS MH+ = 540
7			Glass MS MH+ = 554
8			Solid mp 72-75 °C MS MH+ = 554
9			Solid mp 102-104 °C MS MH+ = 566
10			Solid mp 75-76 °C MS MH+ = 552
11			Solid mp 124-125 °C MS MH+ = 580
12			Solid mp 134-135 °C MS MH+ = 526

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13			Solid mp 78-79 °C MS $MH^+=554$
14			Solid mp 85-86 °C MS $MH^+=580$
15			Solid mp 92-94 °C MS $MH^+=554$
16			Solid mp 115-117 °C MS $MH^+=631$
17			Solid mp 109-110 °C MS $MH^+=617$
18			Solid mp 111-112 °C MS $MH^+=617$
19			Solid mp 77-78 °C MS $MH^+=616$
20			Solid MS $MH^+=650$
21			Solid MS $MH^+=630$
22			Solid mp 66-67 °C MS $MH^+=630$

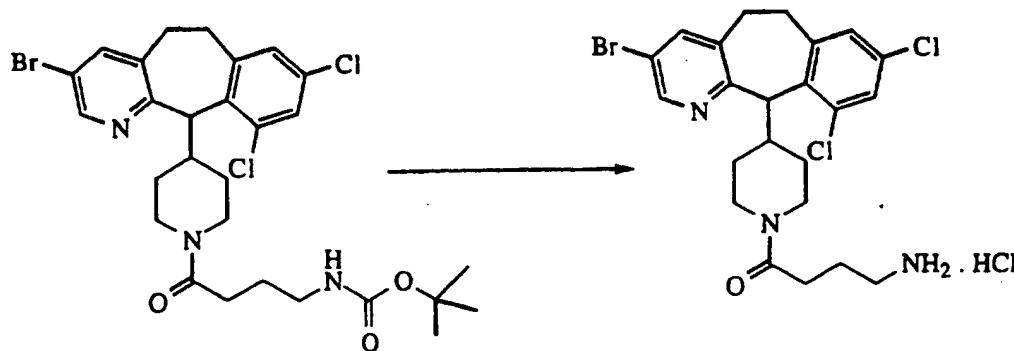
-52-

23			Solid MS MH ⁺ =620
24			Solid mp 130-131 °C MS MH ⁺ =617
25			Solid mp 93-99 °C MS MH ⁺ =612 24 $\alpha_D = 48.2^\circ$, $c=0.23$, CH_2Cl_2
26			Solid mp 103-117 MS MH ⁺ =584
40			Solid mp=72-73°C MS MH ⁺ =541
41			Solid MS MH ⁺ =555

EXAMPLE 27

(+)-1-(4-AMINO-1-OXOBUTYL)-4-(3-BROMO-8,10-DICHLORO-6,11-DIHYDRO-5H-BENZO[5,6]CYCLO-HEPTA[1,2-b]PYRIDIN-11-YL)PIPERIDINE

5



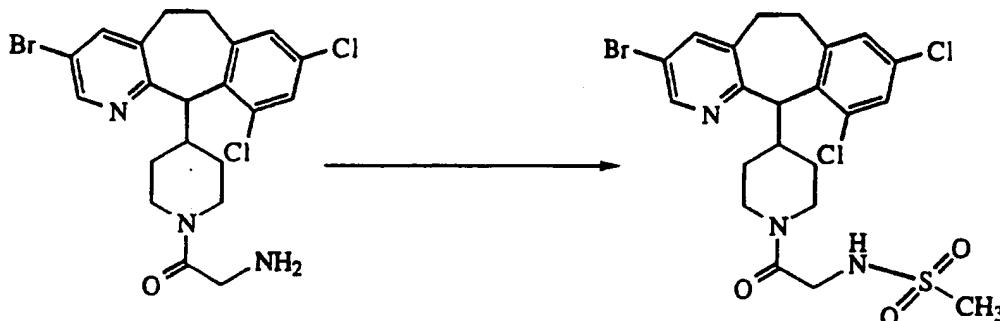
-53-

The title compound is prepared from the title compound of Example 25, following essentially the same procedure as described for Example 3 except that HCl in dioxane was used instead of TFA to obtain the title compounds as a white solid (mp = 112-118 °C, MH⁺ = 512).

5 $\alpha_D^{24} = 64.0^\circ, c = 0.14$, ethanol

EXAMPLE 28

(+)-N-[2-[4-(3-BROMO-8,10-DICHLORO-6,11-DIHYDRO-5H-BENZO[5,6]CYCLO-HEPTA[1,2-b]PYRIDIN-11-YL) PIPERIDINYLYL]-2-OXOETHYL] METHANESULFONAMIDE



The title compound of Example 3 (0.15 g, 0.31 mmol) was dissolved in CH₂Cl₂ (1.5 mL). 4-Methylmorpholine (102 uL), followed by mesyl chloride (36 uL, 0.47 mmol, 1.5 equiv.) was then added. Reaction mixture was stirred at room temperature overnight. The CH₂Cl₂ phase was washed twice with Sat. NaHCO₃, brine and then dried over Na₂SO₄. CH₂Cl₂ was then removed by rotary evaporation and resulting residue was purified on silica gel column eluting with 30% EtOAc/CH₂Cl₂ to give the title compound as a white solid (0.109 g, mp = 120-140 °C, MH⁺ = 562).

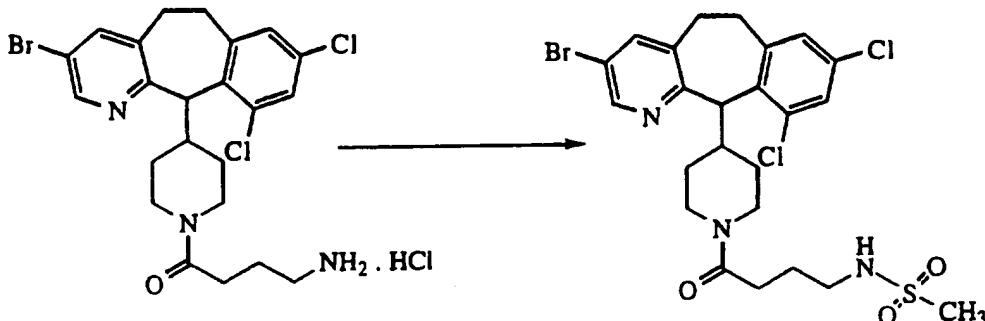
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EXAMPLE 29

(+)-N-[4-[4-(3-BROMO-8,10-DICHLORO-6,11-DIHYDRO-5H-BENZO[5,6]CYCLO-HEPTA[1,2-b]PYRIDIN-11-YL) PIPERIDINYL]-2-OXOBUTYL] METHANESULFONAMIDE



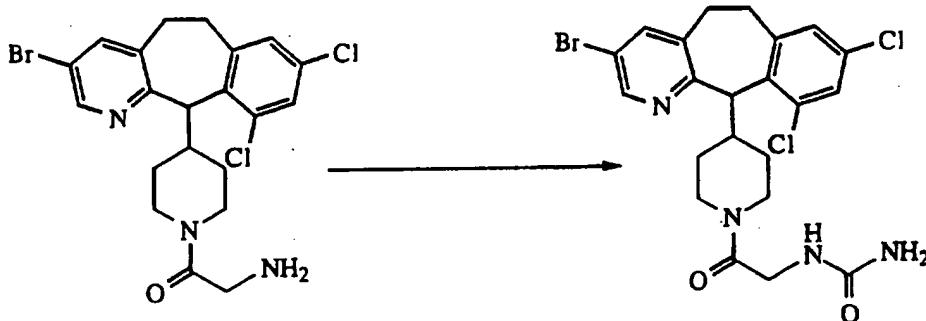
5

The title compound is prepared from the title compound of Example 27, following essentially the same procedure as described for Example 28 to obtain the title compound as a white solid (mp = 110-113 °C, MH⁺ = 590).

10

EXAMPLE 30

(+)-N-[2-[4-(3-BROMO-8,10-DICHLORO-6,11-DIHYDRO-5H-BENZO[5,6]CYCLO-HEPTA[1,2-b]PYRIDIN-11-YL) PIPERIDINYL]-2-OXOETHYL] UREA



15

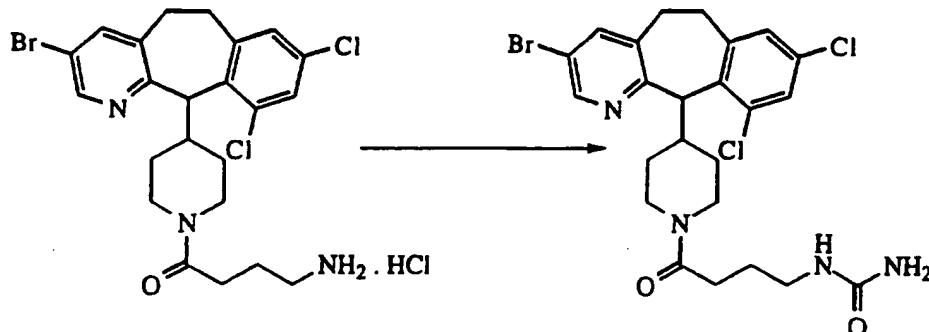
The title compound is prepared from the title compound of Example 3, following essentially the same procedure as described for Example 1 to obtain the title compound as a white solid (MH⁺ = 527)

20

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EXAMPLE 31

(+)-N-[2-[4-(3-BROMO-8,10-DICHLORO-6,11-DIHYDRO-5H-BENZO[5,6]CYCLO-HEPTA[1,2-b]PYRIDIN-11-YL) PIPERIDINYL]-2-OXOBUTYL] UREA

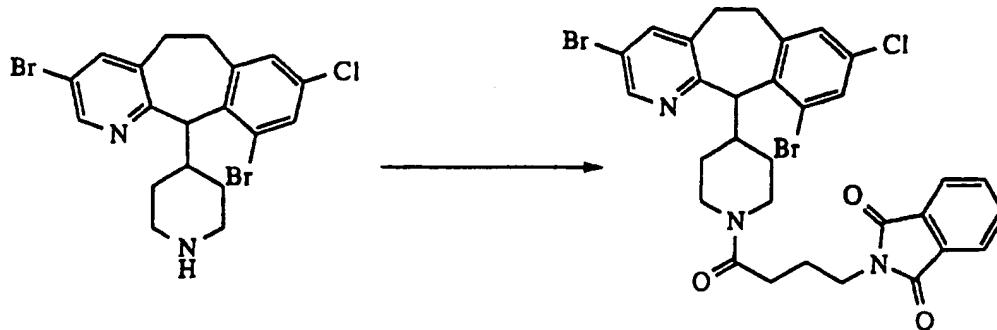


5

The title compound of Example 27 (0.05 g, 0.091 mmol) was dissolved in H₂O (1 mL) and urea (0.055 g, 0.9 mmol) was added. The reaction was heated at ~ 78 °C overnight. Reaction mixture was partitioned between 1N NaOH and CH₂Cl₂. CH₂Cl₂ fraction was dried over MgSO₄ and concentrated. The residue was purified on silica gel on a plate eluting with 5% MeOH (sat. with ammonia)-CH₂Cl₂ eluent to give the title compound as a light yellow powder (MH⁺ = 555, mp = 182-190 °C).

EXAMPLE 32

15 (+)-4-(3,10-DIBROMO-8-CHLORO-6,11-DIHYDRO-5H-BENZO[5,6]CYCLO-HEPTA[1,2-b]PYRIDIN-11-YL)-1-[4-(1,3-DIHYDRO-1,3-DIOXO-2H-ISOINDOL-2-YL)-1-OXOBUTYL]PIPERIDINE



20 The title compound from preparative example 5, Step B (+ enantiomer) (100 mg, 0.21 mmole) was dissolved in 2 mL DMF and 1-hydroxybenzotriazole hydrate (43 mg, 0.32 mmole), 4(1,3-dioxo-1,3-dihydroisoindol-2-yl)butyric acid (0.02 mL, 0.32 mmole), 1-methyl-

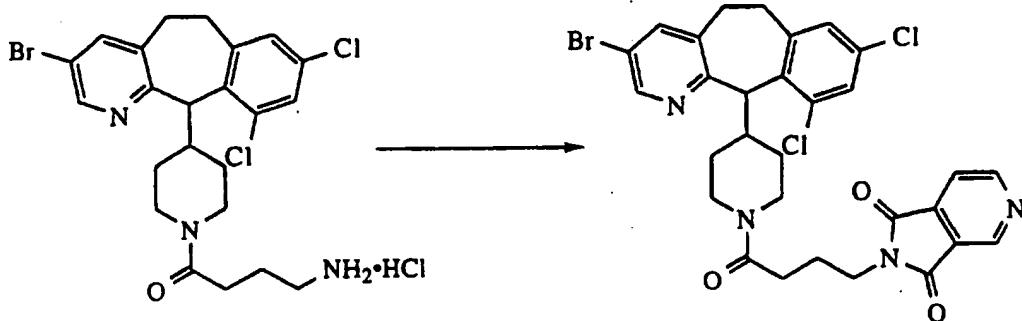
-56-

morpholine (0.04 mL, 0.32 mmole) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride 61 mg, 0.32 mmole) were added. The resulting mixture was stirred at room temperature for 65 h and poured into 10 mL saturated NaHCO₃ solution. The aqueous mixture was
 5 extracted with dichloromethane and the organic solution washed with brine and water, dried with MgSO₄, and evaporated. The resulting residue was purified by silica gel chromatography using 2.5% (ammonia saturated methanol)/dichloromethane as eluent to yield 107 mg of the title compound as a white solid (mp 100.5° - 101.8° C, MH⁺ 686).

10

EXAMPLE 33

4-(8,10-DICHLORO-3-BROMO-6,11-DIHYDRO-5H-BENZO[5,6]CYCLO-HEPTA[1,2-b]PYRIDIN-11-YL)-1-[4-(1,3-DIHYDRO-1,3-DIOXO-2H-PYRROLO-[3,4c]-PYRIDINE-2-YL)-1-OXOBUTYL]PIPERIDINE



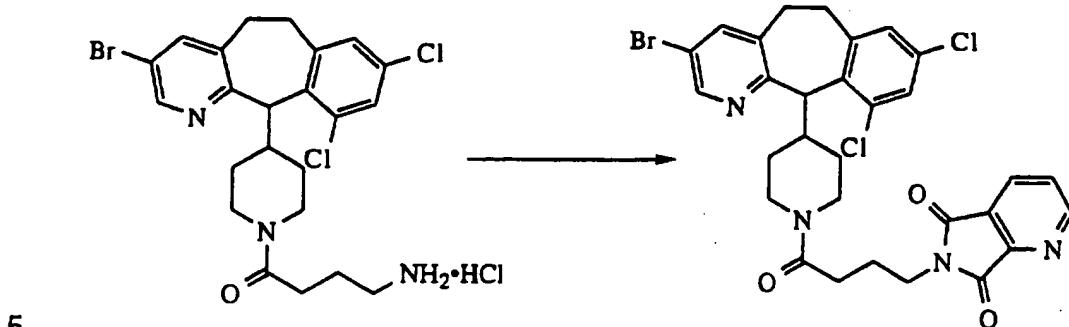
15

The title compound of Example 27 (93.3 mg, 0.171 mmole) was dissolved in 0.75 mL DMF and triethylamine (50 µL, 0.36 mmole) and 3,4-pyridinedicarboxylic anhydride (30.4 mg, 0.204 mmole) were added. The mixture was stirred at room temperature for 4.5 h, heated to 40° to 50° C for 1/2 h then evaporated to dryness. The residue was suspended
 20 in 1 mL acetic anhydride and heated to 85° -95° C for 24 h. The mixture was evaporated to dryness. The residue was dissolved in 2 mL DMF and 0.5 mL water, heated on a steam bath for 1/2 h then added to a stirred solution of NaHCO₃ (170.3 mg) in 10 mL water. The resulting suspension was filtered and the filter cake washed with water then dried
 25 at 50° C under vacuum for 16 h to give 81.9 mg of the title compound as a white solid. mp 105.9-112.2°C, MH⁺ 643.

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EXAMPLE 34

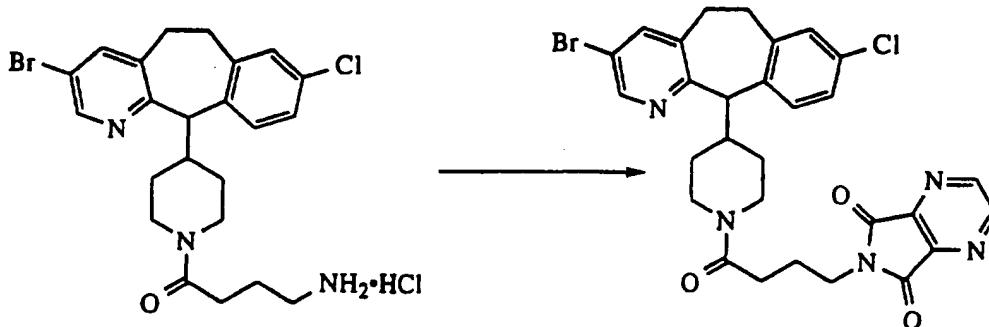
4-(8,10-DICHLORO-3-BROMO-6,11-DIHYDRO-5H-BENZO[5,6]CYCLO-
HEPTA[1,2-b]PYRIDIN-11-YL)-1-[4-(1,3-DIHYDRO-1,3-DIOXO-2H-
PYRROLO-[3,4b]-PYRIDINE-2-YL)-1-OXOBUTYL]PIPERIDINE



The title compound of example 27 (104 mg, 0.191 mmole) was dissolved in 0.75 mL DMF and triethylamine (50 μ L, 0.36 mmole) and 2,3-pyridinedicarboxylic anhydride (301.6 mg, 0.212 mmole) were added. The mixture was stirred at room temperature for 2 h and
 10 evaporated to dryness. The residue was suspended in 1 mL acetic anhydride and heated to 70° to 80° C for 2 hours and evaporated to dryness. The residue was dissolved in 1.5 mL hot DMF and added to a solution of 146 mg NaHCO₃ in 10 mL water. The resulting precipitate was filtered, washed with water and vacuum dried at 50°C for 16 h to
 15 afford 74.0 mg of the title compound as a white solid (mp 126.0°-
 135.2°C, heating 2 to 3°C per minute), MH⁺ 643.

EXAMPLE 35

4-(8,10-DICHLORO-3-BROMO-6,11-DIHYDRO-5H-BENZO[5,6]CYCLO-
HEPTA[1,2-b]PYRIDIN-11-YL)-1-[4-(1,3-DIHYDRO-1,3-DIOXO-2H-
 20 PYRROLO-[3,4b]-PYRAZINE-2-YL)-1-OXOBUTYL]PIPERIDINE

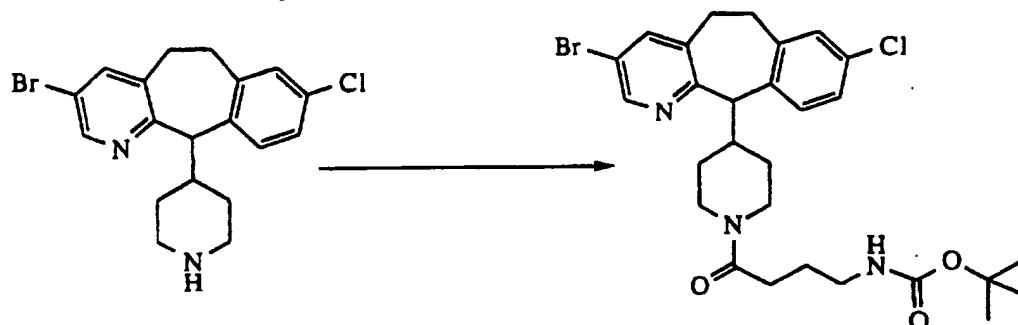


-58-

The procedure of Example 33 was followed using 100.0 mg (0.19 mmole) of the title compound of Example 37 44.0 mg (0.241 mmole) 2,3-pyrazinedicarboxylic anhydride and 55 μ L triethylamine in 0.75 mL DMF. Following the procedure described in Example 33 the title 5 compound was obtained as a white solid mp 124.0-125.5°C, MH^+ 610.

EXAMPLE 36

(+/-)-1-(5-AZA-8,8 DIMETHYL-1,6-DIOXO-7-OXYNONYL)-4-(3-BROMO-8-CHLORO-6,11-DIHYDRO-5H-BENZO[5,6]CYCLOHEPTA-[1,2b]PYRIDIN-11-YL)PIPERIDINE



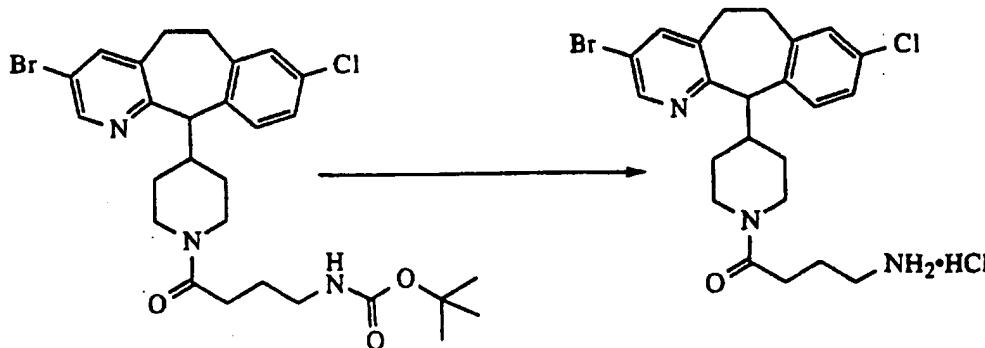
10

The title compound is prepared from the title compound of preparative example 8 following essentially the same procedure as described for the preparation of the starting material of Example 27 (mp = 90.3 - 93.4 °C, $MH^+ = 578$).

15

EXAMPLE 37

(+/-)-1-(4-AMINO-1-OXOBUTYL)-4-(3-BROMO-8-CHLORO-6,11-DIHYDRO-5H-BENZO[5,6]CYCLOHEPTA[1,2b]PYRIDIN-11-YL)PIPERIDINE



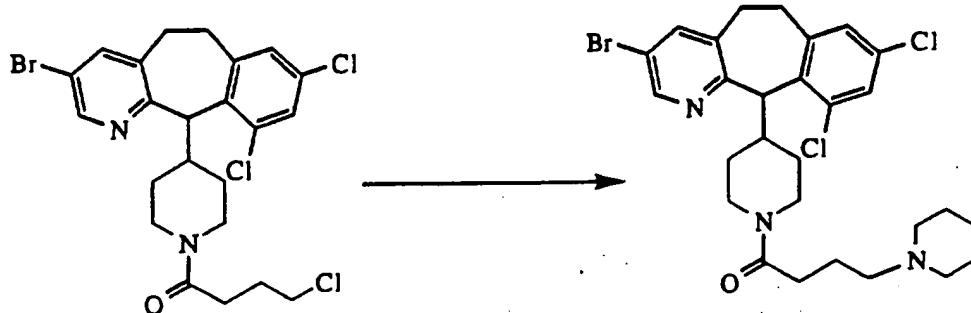
20

The title compound is prepared from the title compound of Example 36, following essentially the same procedure as described for Example 27 to obtain the title compound as a pale yellow solid (mp 50.8 - 55.5 °C), $MH^+ 478$.

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EXAMPLE 38

(+)-4-(3-BROMO-8,10-DICHLORO-6,11-DIHYDRO-5H-BENZO[5,6]CYCLO-HEPTA[1,2-b]PYRIDIN-11-YL)-1-[4-(PIPERIDINYLPYRIPIDINE)-1-OXOBUTYL] PIPIRIDINE



5

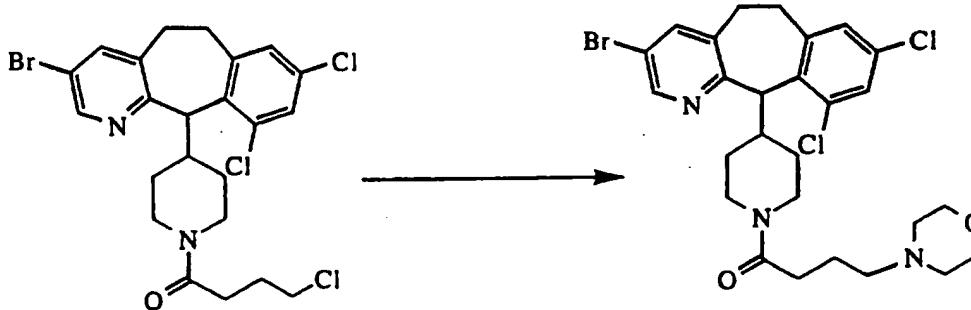
The title compound of Preparative Example 3 (0.1g, 0.17 mmol) and pipiridine(0.1 mL, 1.04 mmol) were dissolved in 5 mL of CH_2Cl_2 and stirred at room temperature for 48h. All the volatile solvents were removed and the resulting crude product was purified on a silica gel prep plate eluting with 20% $\text{MeOH-NH}_3 - \text{CH}_2\text{Cl}_2$ to give 0.02g of the title compound FAB-MS: $\text{MH}^+ = 580$

10

15

EXAMPLE 39

(+)-4-(3-BROMO-8,10-DICHLORO-6,11-DIHYDRO-5H-BENZO[5,6]CYCLO-HEPTA[1,2-b]PYRIDIN-11-YL)-1-[4-(MORPHOLINYLPYRIPIDINE)-1-OXOBUTYL] PIPIRIDINE



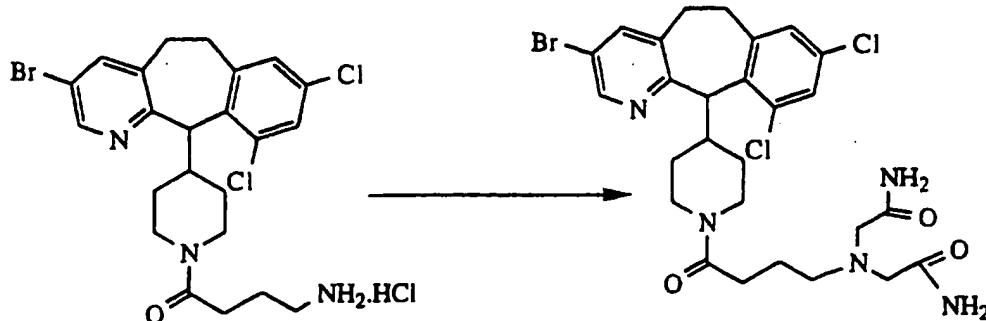
15

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The title compound is prepared following essentially the same procedure as described in Example 38 except that morpholine was used instead of pipiridine to obtain a solid FAB-MS: $\text{MH}^+ = 582$.

-60-

EXAMPLE 42

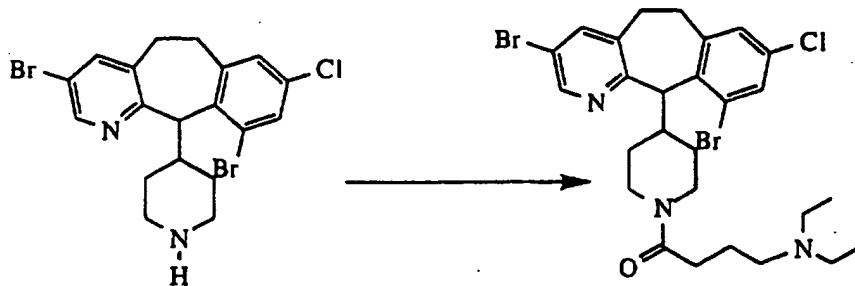


The title compound of Example 27 (0.1 g, 0.18 mmole),
bromoacetamide (0.04 g, 0.3 mmole) and potassium carbonate were
5 dissolved in 2 ml of DMF and allowed to stand for 18 hours. The reaction
mixture was concentrated in vacuo. The residue was dissolved in ethyl
acetate, washed with water, dried over magnesium sulfate, and
concentrated to give a white solid. mp=110°C to 123°C, MH=626,

$$\alpha_D^{24} = +30.6^{\circ}, \quad c = 0.17, \text{CH}_2\text{Cl}_2.$$

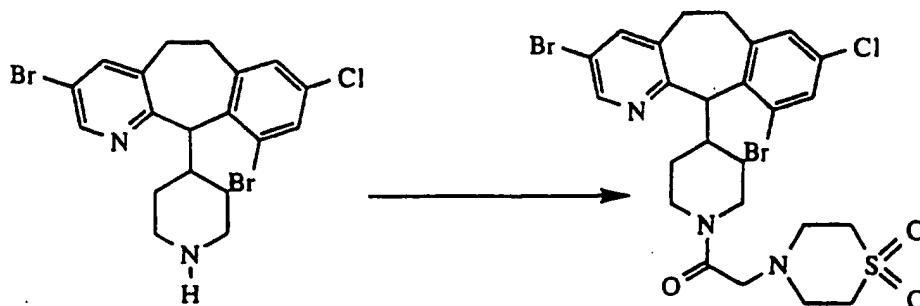
10

EXAMPLE 43



The title compound was prepared by essentially the same
procedure as described in Example 2 except that 4-(diethylamino)butyric
acid was used in place of 4-(methylamino)butyric acid, mp= 69.9-
15 70.1°C.

EXAMPLE 44

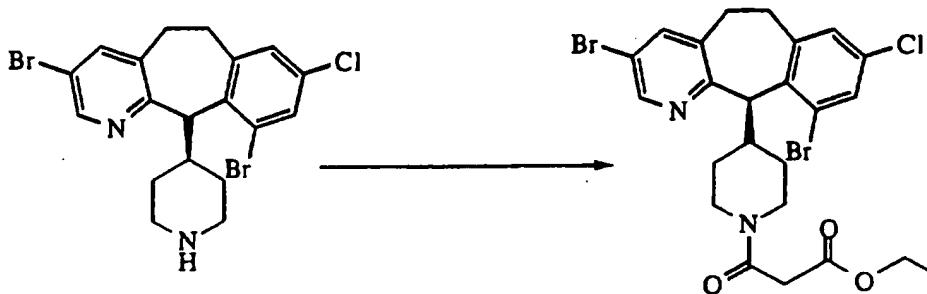


-61-

The title compound was prepared by essentially the same procedure as described in Example 2 except that 4-(diethylamino)butyric acid was replaced with thiomorpholine S-dioxide acetic acid.

EXAMPLE 45

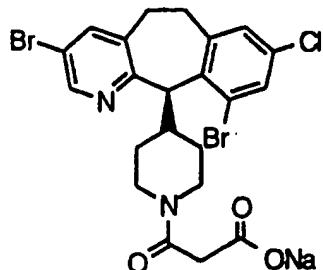
5 (+)-ETHYL 4-(3,10-DIBROMO-8-CHLORO-6,11-DIHYDRO-5H-BENZO[5,6]CYCLO-HEPTA[1,2-b]PYRIDIN-11(R)-YL)-BETA-OXO -1-PIPERIDININE PROPAANOATE



The product of Preparative Example 5, Step B -(+isomer) (0.4 g, 0.85 mmol), was dissolved in DMF (10 mL) and then cooled to ~ 4°C. Mono ethyl malonate potassium salt (0.19 g, 1.1 mmol) was then added, followed by DEC (0.2 g, 1.1 mmol), HOBT (0.15 g, 1.1 mmol), and 4-methylmorpholine (0.11 g, 0.12 µL, 1.1 mmol). The reaction was then stirred at room temperature overnight. The reaction mixture was concentrated in vacuo to a residue that was partitioned between CH₂Cl₂ and sat. NaHCO₃ (aqueous). The aqueous phase was extracted further with CH₂Cl₂, the combined CH₂Cl₂ fractions were dried over MgSO₄ and concentrated in vacuo. The resultant residue was chromatographed on a silica gel column using 50 % EtOAc-Hexanes as eluent to give the title compound as a white solid (0.41 g, 82% yield, m.p.=86-87°C, M⁺=585).

EXAMPLE 46

(+)-SODIUM 4-(3,10-DIBROMO-8-CHLORO-6,11-DIHYDRO-5H-BENZO[5,6]CYCLO-HEPTA[1,2-b]PYRIDIN-11(R)-YL)-BETA-OXO -1-PIPERIDININE PROPAANOATE

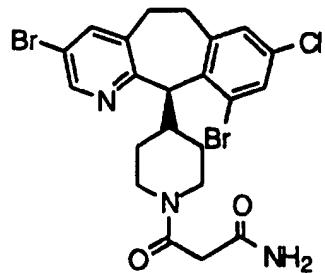


-62-

The product of Example 45 (0.34 g, 0.58 mmol) was dissolved in absolute EtOH (10 mL). H₂O (0.7 mL) was then added, followed by NaOH (0.03 g, 0.7 mmol). The reaction mixture was stirred at room temperature for 16h. The solvents were removed by rotary evaporation
 5 to give the title compound as a white solid (0.34 g, 100% yield, m.p.=230°C (decomposed), M^{H+}=556).

EXAMPLE 47

10 4-(3,10-DIBROMO-8-CHLORO-6,11-DIHYDRO-5H-BENZO[5,6]CYCLO-
 HEPTA[1,2-b]PYRIDIN-11(R)-YL)-BETA-OXO -1-PIPERIDININE
 PROPANAMIDE

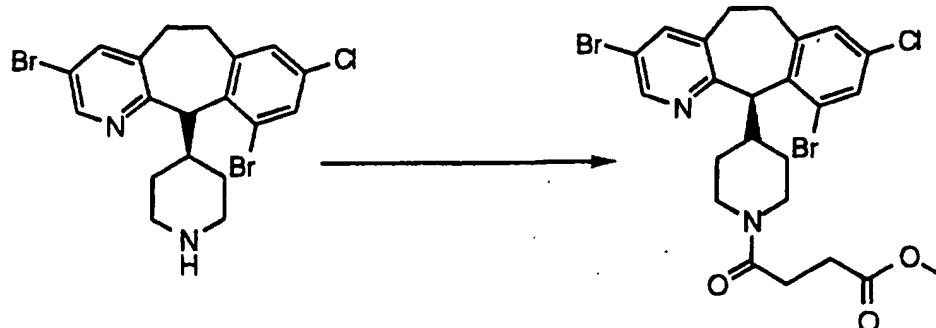


The product of Example 46 (0.4 g, 0.72 mmol), was dissolved in DMF (10mL) and then cooled to ~ 4°C. NH₄Cl (0.05 g, 0.94 mmol) was added, followed by DEC (0.17 g, 0.94 mmol), HOBT (0.13 g, 0.94 mmol), and 4-methylmorpholine (0.09 g, 0.1 μL, 0.094 mmol). The reaction was then stirred at room temperature overnight. The reaction mixture was concentrated in vacuo to a residue which was partitioned between CH₂Cl₂ and sat. NaHCO₃ (aqueous). The aqueous phase was extracted further with CH₂Cl₂, the combined CH₂Cl₂ fractions were dried over MgSO₄ and concentrated in vacuo. The resultant residue was chromatographed on a silica gel column using 50 % EtOAc-Hexanes as eluent to give the title compound as a white solid (0.22 g, 55% yield, m.p.=143-144°C,
 15 M^{H+}=556).
 20
 25

-63-

EXAMPLE 48

(+)-METHYL 4-(3,10-DIBROMO-8-CHLORO-6,11-DIHYDRO-5H-BENZO[5,6]CYCLO-HEPTA[1,2-b]PYRIDIN-11(R)-YL)-GAMMA-OXO -1-PIPERIDININE BUTANOATE



5

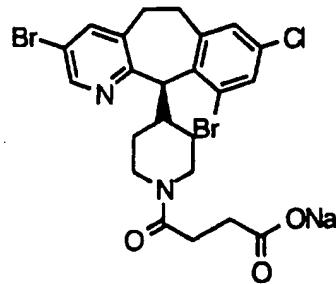
The title compound is prepared following essentially the same procedure as described for Example 45, using the appropriate diacid monoester to obtain the title compound as a white solid (yield = 72 %, m.p.=78-79°C, $\text{MH}^+=584$).

10

EXAMPLE 49

(+)-SODIUM 4-(3,10-DIBROMO-8-CHLORO-6,11-DIHYDRO-5H-BENZO[5,6]CYCLO-HEPTA[1,2-b]PYRIDIN-11(R)-YL)-GAMMA-OXO -1-PIPERIDININE BUTANOATE

15



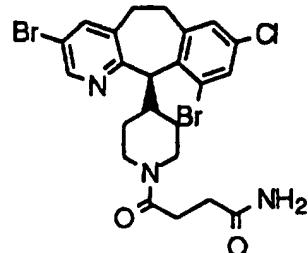
The title compound is prepared from the product of Example 48 following essentially the same procedure as described for Example 46 to obtain the title compound as a white solid (94% yield, m.p.=270°C (decomposed), $\text{MH}^+ = 570$).

20

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EXAMPLE 50

(+)-4-(3,10-DIBROMO-8-CHLORO-6,11-DIHYDRO-5H-BENZO[5,6]CYCLO-HEPTA[1,2-b]PYRIDIN-11(R)-YL)-GAMMA-OXO -1-PIPERIDININE BUTANAMIDE



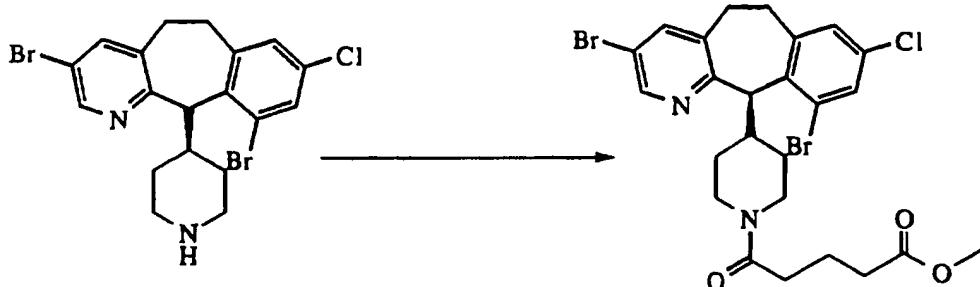
5

The title compound is prepared from the product of Example 49 following essentially the same procedure as described for Example 47 to obtain a white solid (yield = 45%, m.p.=134-135°C, MH^+ = 570).

10

EXAMPLE 51

(+)-METHYL 4-(3,10-DIBROMO-8-CHLORO-6,11-DIHYDRO-5H-BENZO[5,6]CYCLO-HEPTA[1,2-b]PYRIDIN-11(R)-YL)-DELTA-OXO -1-PIPERIDININE PENTANOATE



15

The title compound is prepared following essentially the same procedure as described for Example 45 using the appropriate diacid monoester to obtain the title compound as a white solid (yield = 94 %, m.p.=74-75°C, MH^+ = 599).

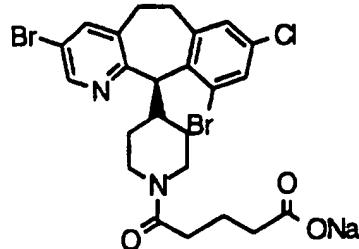
20

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-65-

EXAMPLE 52

(+)-SODIUM 4-(3,10-DIBROMO-8-CHLORO-6,11-DIHYDRO-5H-BENZO[5,6]CYCLO-HEPTA[1,2-b]PYRIDIN-11(R)-YL)-DELTA-OXO -1-PIPERIDININE PENTANOATE



5

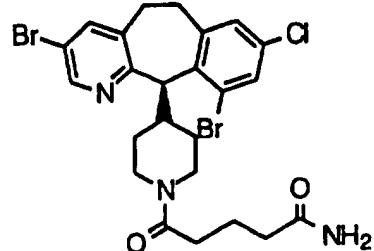
The title compound is prepared from the product of Example 51 following essentially the same procedure as described for Example 46 to obtain the title compound as a white solid (93% yield, m.p.=282°C (decomposed), $MH^+ = 584$).

10

EXAMPLE 53

(+)- 4-(3,10-DIBROMO-8-CHLORO-6,11-DIHYDRO-5H-BENZO[5,6]CYCLO-HEPTA[1,2-b]PYRIDIN-11(R)-YL)-DELTA-OXO -1-PIPERIDININE PENTANAMIDE

15



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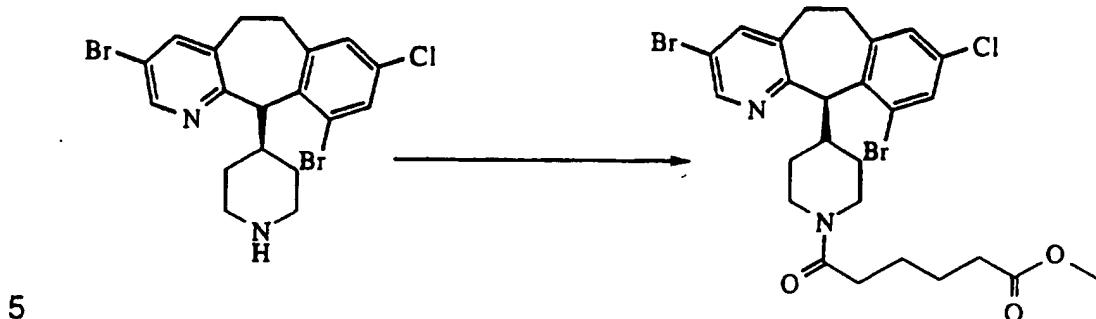
The title compound is prepared form the product of Example 52 following essentially the same procedure as described for Example 47 to obtain the title compound as a white solid (yield = 61 %, m.p.=124-125°C, $MH^+ = 584$).

25

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EXAMPLE 54

(+)-METHYL 4-(3,10-DIBROMO-8-CHLORO-6,11-DIHYDRO-5H-BENZO[5,6]CYCLO-HEPTA[1,2-b]PYRIDIN-11(R)-YL)-EPSILON-OXO-1-PIPERIDININE HEXANOATE



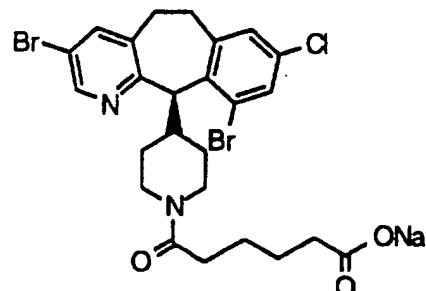
The title compound is prepared following essentially the same procedure as described for Example 45 using the appropriate diacid monoester to obtain the title compound as a white solid (yield = 92 %, m.p.=84-85°C, $MH^+ = 613$).

10

EXAMPLE 55

(+)-SODIUM 4-(3,10-DIBROMO-8-CHLORO-6,11-DIHYDRO-5H-BENZO[5,6]CYCLO-HEPTA[1,2-b]PYRIDIN-11(R)-YL)-EPSILON-OXO-1-PIPERIDININE HEXANOATE

15

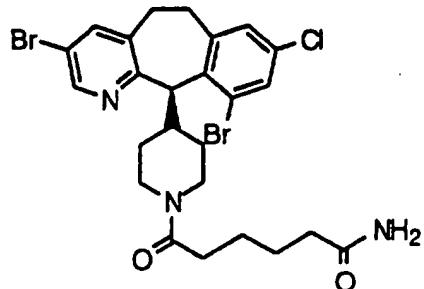


The title compound is prepared from the product of Example 54 following essentially the same procedure as described for Example 46 to obtain the title compound as a white solid (97% yield, m.p.=135-136°C, 20 $MH^+ = 598$).

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EXAMPLE 56

(+)-4-(3,10-DIBROMO-8-CHLORO-6,11-DIHYDRO-5H-BENZO[5,6]CYCLO-HEPTA[1,2-b]PYRIDIN-11(R)-YL)-EPSILON-OXO-1-PIPERIDININE HEXANAMIDE



5

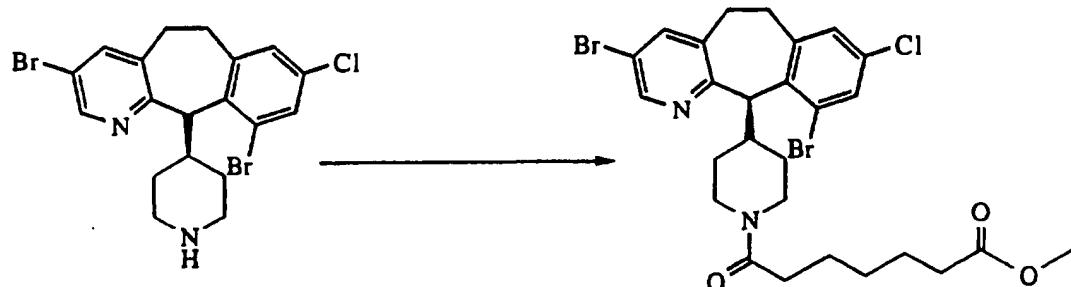
The title compound is prepared from the product of Example 55 following essentially the same procedure as described for Example 47 to obtain the title compound as a white solid (38% yield, m.p.=119-120°C, $MH^+ = 598$).

10

EXAMPLE 57

(+)-METHYL 4-(3,10-DIBROMO-8-CHLORO-6,11-DIHYDRO-5H-BENZO[5,6]CYCLO-HEPTA[1,2-b]PYRIDIN-11(R)-YL)-OMEGA-OXO -1-PIPERIDININE HEPTANOATE

15



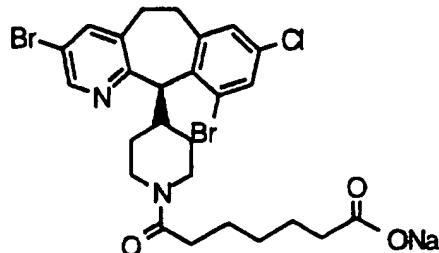
20

The title compound is prepared following essentially the same procedure as described for Example 45, using the appropriate diacid monoester to obtain the title compound as an oil (yield = 97 %, $MH^+ = 627$).

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EXAMPLE 58

(+)-SODIUM 4-(3,10-DIBROMO-8-CHLORO-6,11-DIHYDRO-5H-BENZO[5,6]CYCLO-HEPTA[1,2-b]PYRIDIN-11(R)-YL)-OMEGA-OXO -1-PIPERIDININE HEPTANOATE



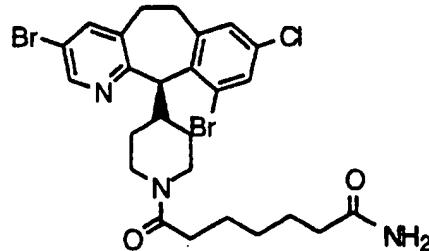
5

The title compound is prepared from the product of Example 57 following essentially the same procedure as described for Example 46 to obtain the title compound as a white solid (yield = 82%, m.p.=142-143°C, $MH^+ = 613$).

10

EXAMPLE 59

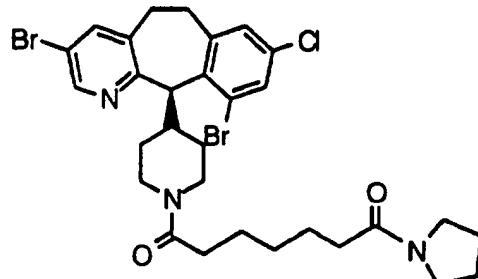
(+)-4-(3,10-DIBROMO-8-CHLORO-6,11-DIHYDRO-5H-BENZO[5,6]CYCLO-HEPTA[1,2-b]PYRIDIN-11(R)-YL)-OMEGA-OXO -1-PIPERIDININE HEPTANAMIDE



15

The title compound is prepared from the product of Example 58 following essentially the same procedure as described for Example 47 to obtain the title compound as a white solid (yield = 30%, m.p.=96-97°C, $MH^+ = 612$).

EXAMPLE 60

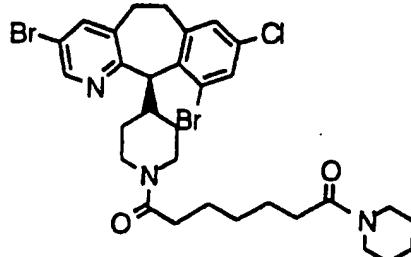


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The title compound is prepared from the product of Example 58 following essentially the same procedure as described for Example 47, using the appropriate amine to obtain the title compound.

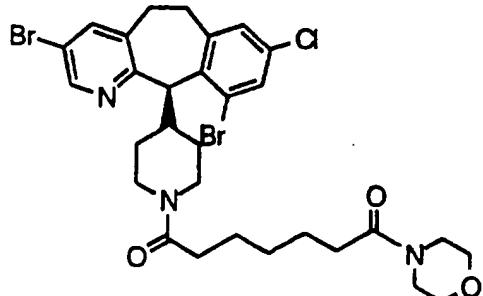
EXAMPLE 61



5

The title compound is prepared from the product of Example 58 following essentially the same procedure as described for Example 47, using the appropriate amine to obtain the title compound.

EXAMPLE 62



10

The title compound is prepared from the product of Example 58 following essentially the same procedure as described for Example 47, using the appropriate amine to obtain the title compound.

Pharmaceutical Dosage Form Examples

EXAMPLE A
Tablets

No.	Ingredients	mg/tablet	mg/tablet
1.	Active compound	100	500
2.	Lactose USP	122	113
3.	Corn Starch, Food Grade, as a 10% paste in Purified Water	30	40
4.	Corn Starch, Food Grade	45	40
5.	Magnesium Stearate	3	7
Total		300	700

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Method of Manufacture

Mix Item Nos. 1 and 2 in a suitable mixer for 10–15 minutes.

Granulate the mixture with Item No. 3. Mill the damp granules through a coarse screen (e.g., 1/4", 0.63 cm) if necessary. Dry the damp granules.

5 Screen the dried granules if necessary and mix with Item No. 4 and mix for 10–15 minutes. Add Item No. 5 and mix for 1–3 minutes. Compress the mixture to appropriate size and weigh on a suitable tablet machine.

EXAMPLE B

Capsules

No.	Ingredient	mg/capsule	mg/capsule
1.	Active compound	100	500
2.	Lactose USP	106	123
3.	Corn Starch, Food Grade	40	70
4.	Magnesium Stearate NF	7	7
	Total	253	700

10 Method of Manufacture

Mix Item Nos. 1, 2 and 3 in a suitable blender for 10-15 minutes.

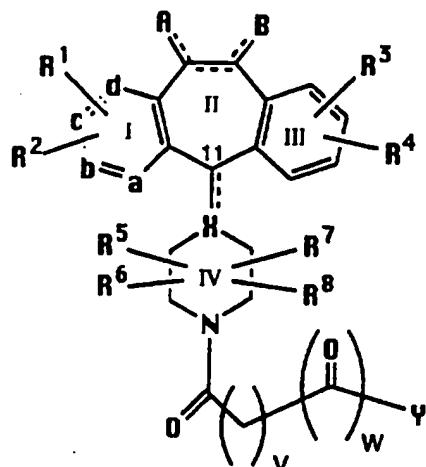
Add Item No. 4 and mix for 1-3 minutes. Fill the mixture into suitable two-piece hard gelatin capsules on a suitable encapsulating machine.

15 While the present invention has been described in conjunction with the specific embodiments set forth herein, many alternatives, modifications and variations thereof will be apparent to those of ordinary skill in the art. All such alternatives, modifications and variations are intended to fall within the spirit and scope of the present invention.

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We Claim:

1. A compound of the formula



5

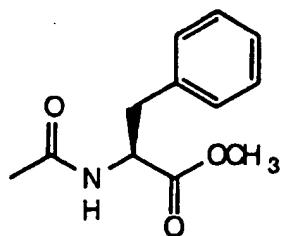
wherein:

X is N, CH, or C when the double bond is present at the C-11 position;

one of a, b, c and d represents N or NR⁹ wherein R⁹ is O⁻, -CH₃ or 10 -(CH₂)_nCO₂H wherein n is 1 to 3, and the remaining a, b, c and d groups represent CR¹ or CR²; or

each of a, b, c, and d are independently selected from CR¹ or CR²;

each R¹ and each R² is independently selected from H, halo, 15 -CF₃, -OR¹⁰ (e.g., -OCH₃), -COR¹⁰, -SR¹⁰ (e.g., -SCH₃ and -SCH₂C₆H₅), -S(O)_tR¹¹ (wherein t is 0, 1 or 2, e.g., -SOCH₃ and -SO₂CH₃), -SCN, -N(R¹⁰)₂, -NR¹⁰R¹¹, -NO₂, -OC(O)R¹⁰, -CO₂R¹⁰, -OCO₂R¹¹, -CN, -NHC(O)R¹⁰, -NHSO₂R¹⁰, -CONHR¹⁰, -CONHCH₂CH₂OH, -NR¹⁰COOR¹¹,



20

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-SR¹¹C(O)OR¹¹ (e.g., -SCH₂CO₂CH₃), -SR¹¹N(R¹²)₂ wherein each R¹² is independently selected from H and -C(O)OR¹¹ (e.g., -S(CH₂)₂NHC(O)O-t-butyl and -S(CH₂)₂NH₂), benzotriazol-1-yloxy, tetrazol-5-ylthio, or substituted tetrazol-5-ylthio (e.g., alkyl substituted tetrazol-5-ylthio such as 1-methyl-tetrazol-5-ylthio), alkynyl, alkenyl or alkyl, said alkyl or alkenyl group optionally being substituted with halo, -OR¹⁰ or -CO₂R¹⁰;

5 R³ and R⁴ are the same or different and each independently represents H, any of the substituents of R¹ and R², or R³ and R⁴ taken together represent a saturated or unsaturated C₅-C₇ fused ring to the benzene ring (Ring III);

10 R⁵, R⁶, R⁷ and R⁸ each independently represents H, -CF₃, -COR¹⁰, alkyl or aryl, said alkyl or aryl optionally being substituted with -OR¹⁰, -SR¹⁰, -S(O)_tR¹¹, -NR¹⁰COOR¹¹, -N(R¹⁰)₂, -NO₂, -COR¹⁰, -OCOR¹⁰, -OCO₂R¹¹, -CO₂R¹⁰, OPO₃R¹⁰, or R⁵ is combined with R⁶ to represent =O or =S and/or R⁷ is combined with R⁸ to represent =O or =S;

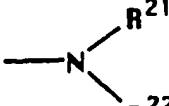
15 R¹⁰ represents H, alkyl, aryl, or aralkyl;

20 R¹¹ represents alkyl or aryl;

20 the dotted line between carbon atoms 5 and 6 represents an optional double bond, such that when a double bond is present, A and B independently represent -R¹⁰, halo, -OR¹¹, -OCO₂R¹¹ or -OC(O)R¹⁰, and when no double bond is present between carbon atoms 5 and 6, A and B each independently represent H₂, -(OR¹¹)₂; H and halo, dihalo, alkyl and H, (alkyl)₂, -H and -OC(O)R¹⁰, H and -OR¹⁰, =O, aryl and H, =NOR¹⁰ or -O-(CH₂)_p-O- wherein p is 2, 3 or 4;

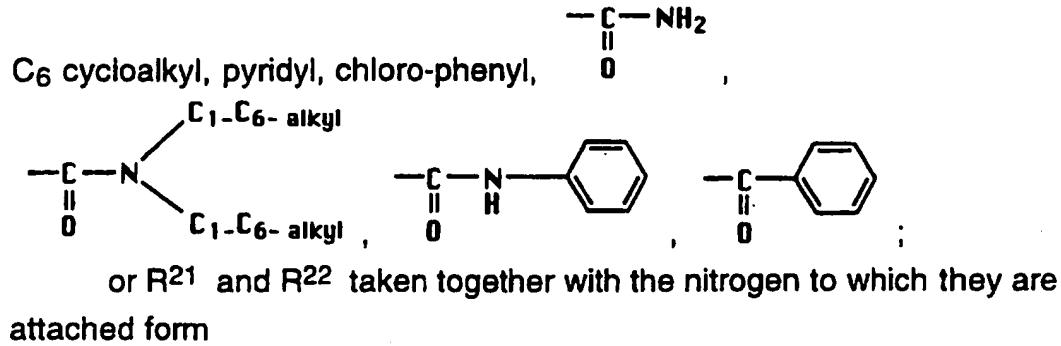
25 v is 0 to 5;

25 w is 0 or 1;

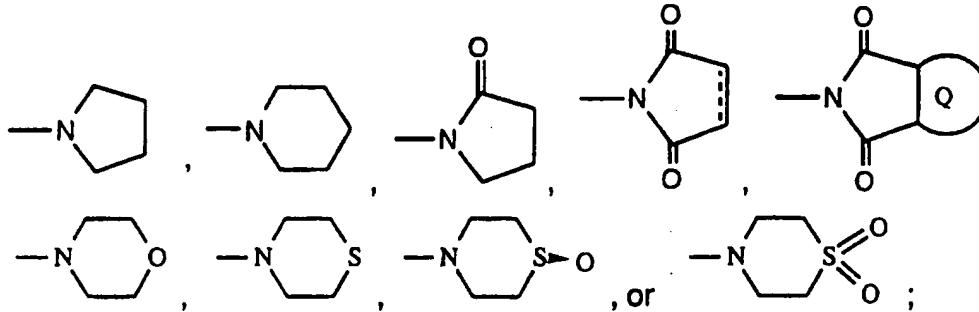
30 Y is  , -O-C₁-C₆-alkyl or -OM+ , wherein M+ is an alkali metal cation;

30 R²¹ and R²² are each independently H, C₁-C₆ alkyl, -CH₂CONH₂, phenyl, benzyl, -SO₂-(C₁-C₆-alkyl), -NH-phenyl, acyl, C₃-

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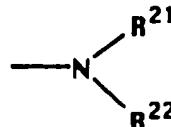
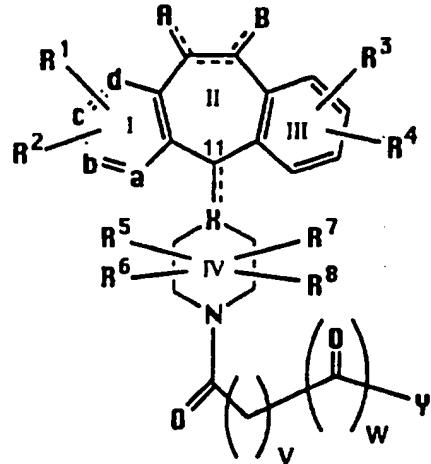
5



wherein Q is benzene or a heterocyclic ring;
 or a pharmaceutically acceptable salt thereof.

10

2. A compound according to claim 1, of the formula



wherein v is 0 to 4; w is 0; and Y is

-74-

3. A compound according to claim 1 wherein a is N, and R¹, R², R³ and R⁴ are each independently selected from the group consisting of H or halo.

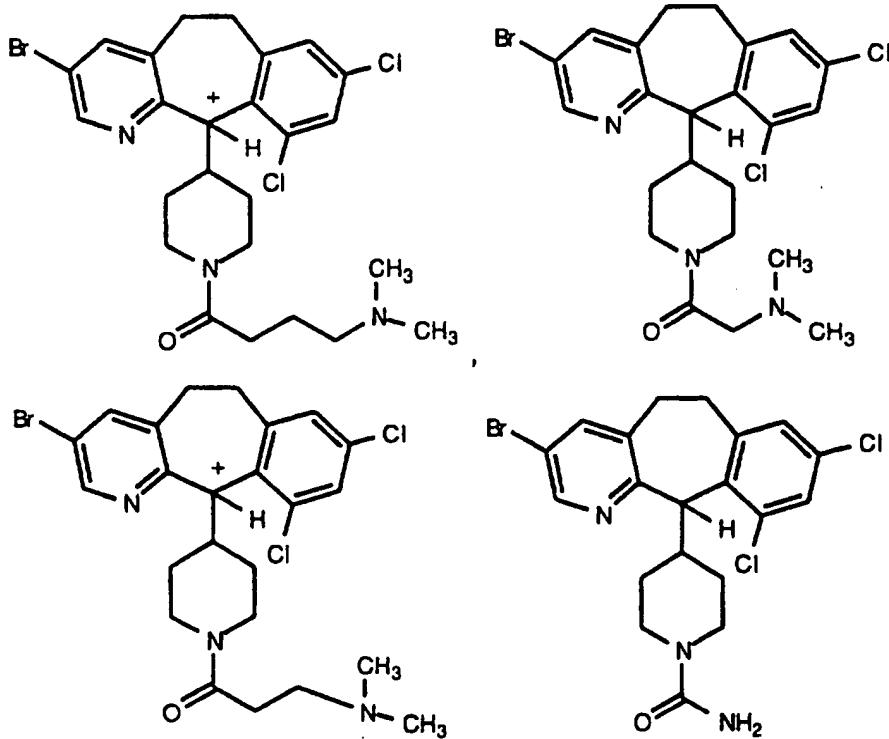
5 4. A compound according to claim 3 wherein R¹ is H; R² is Br; and R³ and R⁴ are each independently selected from the group consisting of Br and Cl.

10 5. A compound according to claim 4 wherein X is CH.

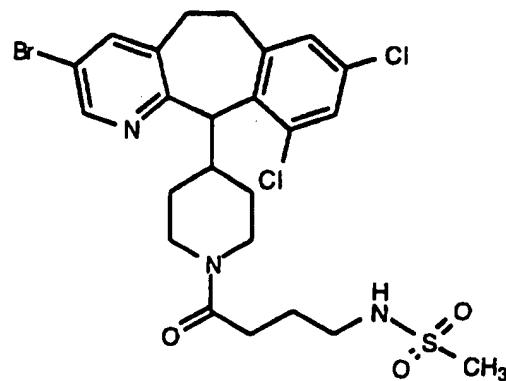
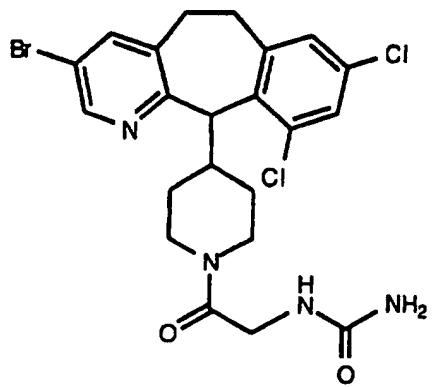
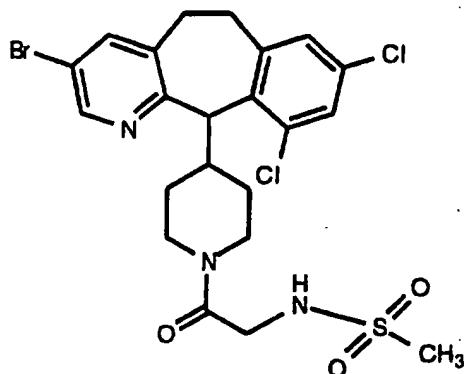
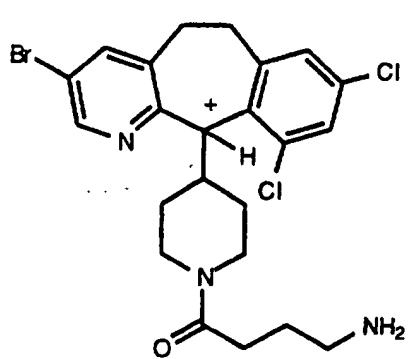
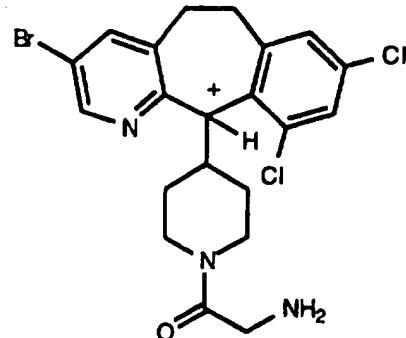
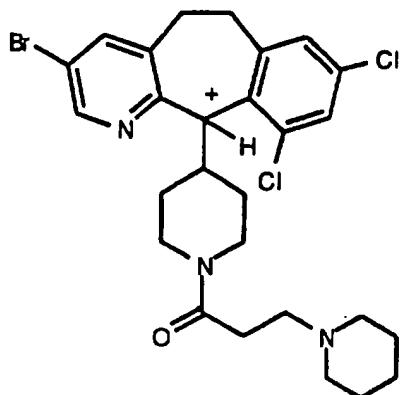
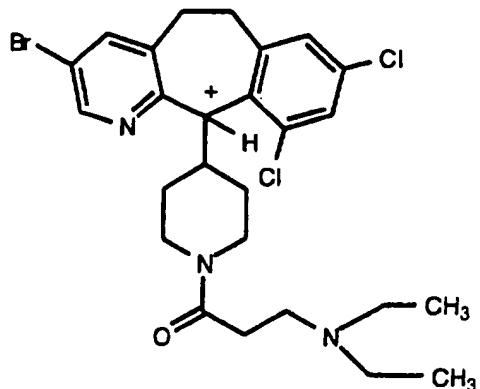
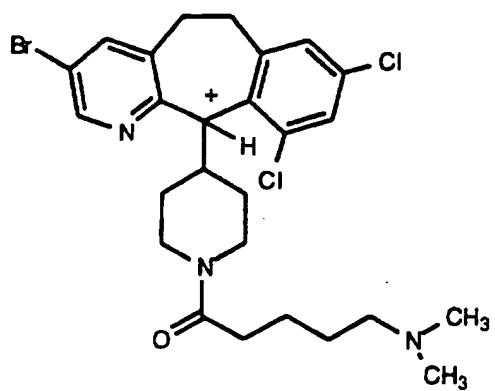
6. A compound according to claim 5 wherein R³ is Cl and R⁴ is Br.

15 7. A compound of claim 1 wherein w is 1; v is 0-5; R¹ is H; R² is Br; R³ and R⁴ are independently Cl and Br; R⁵-R⁸ are each H; X is CH; and Y is -O-C₁-C₆ alkyl, NH₂ or -OM+.

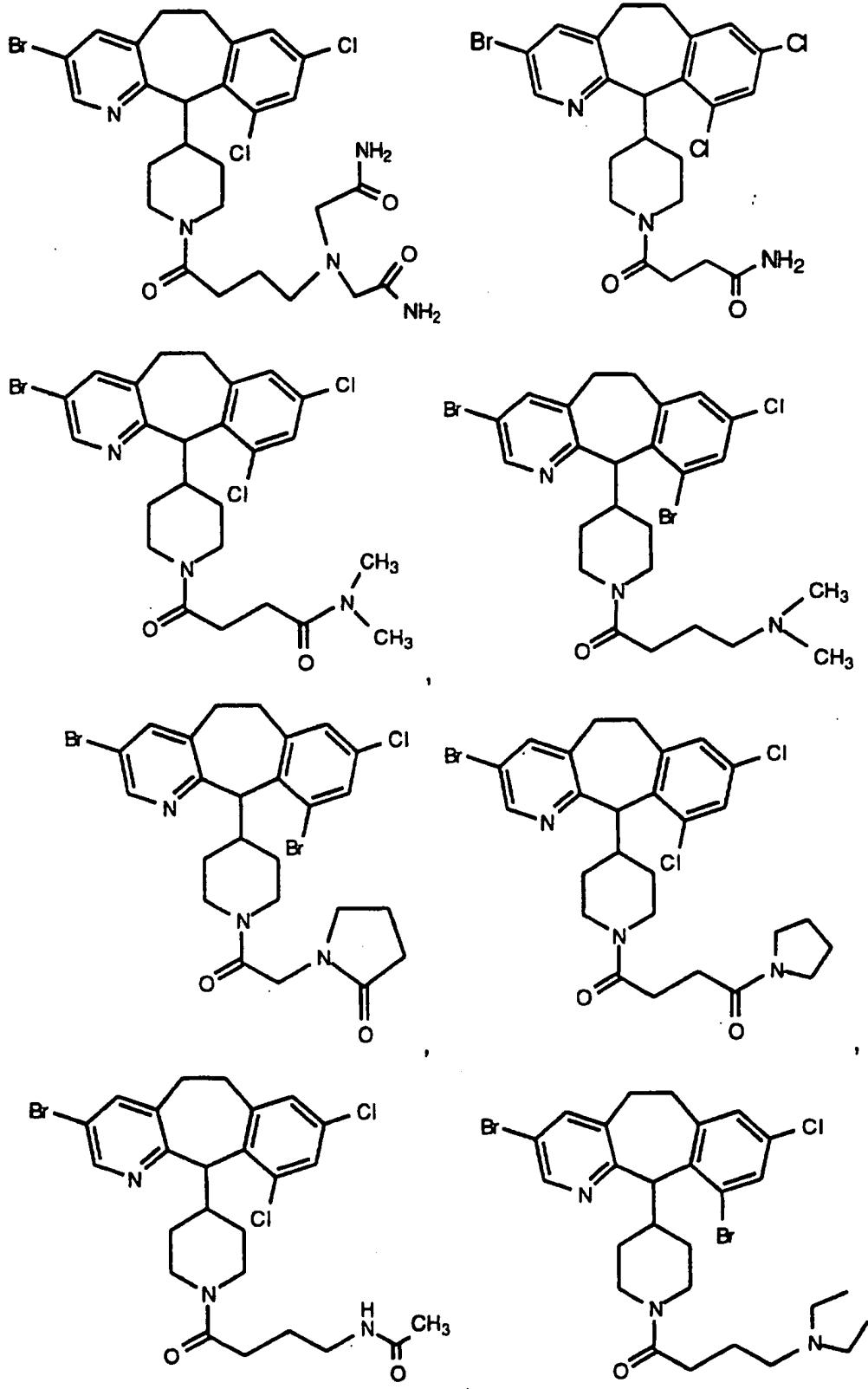
8. A compound according to claim 1 selected from the group consisting of



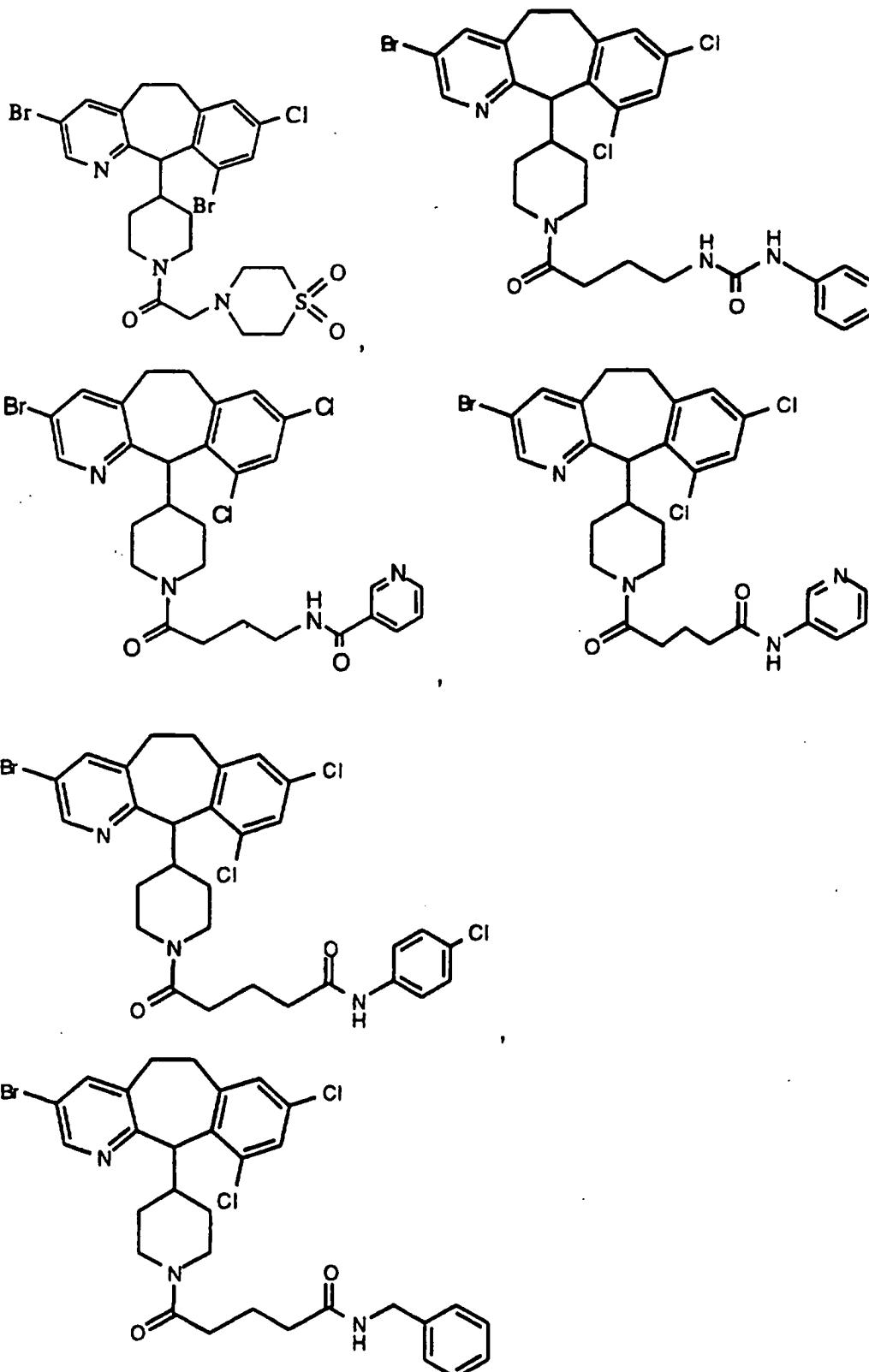
-75-



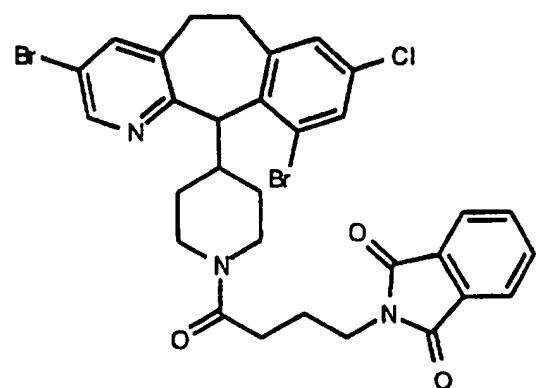
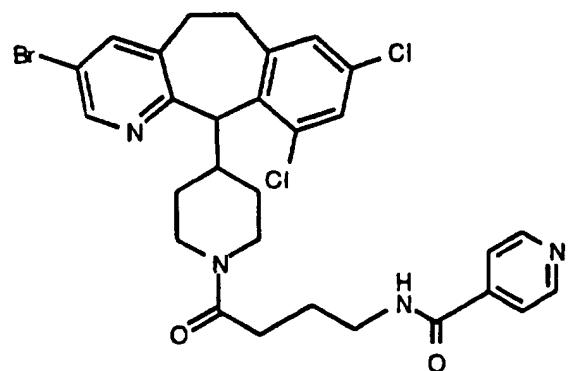
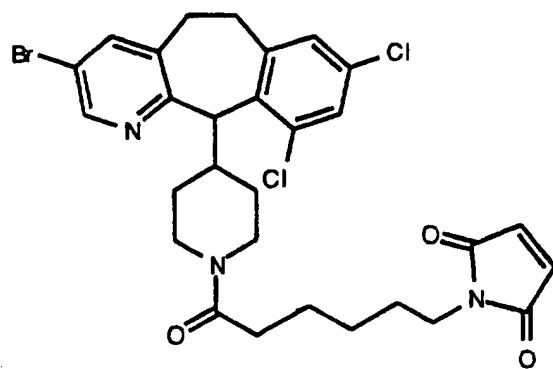
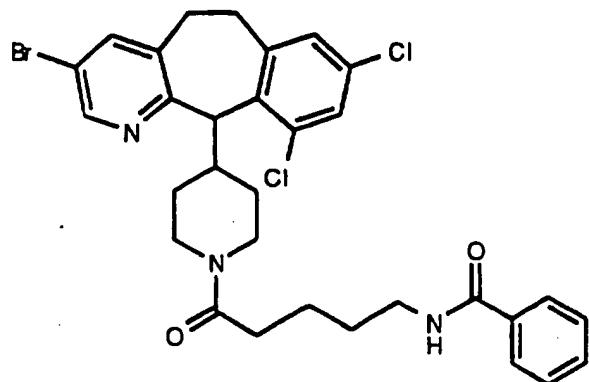
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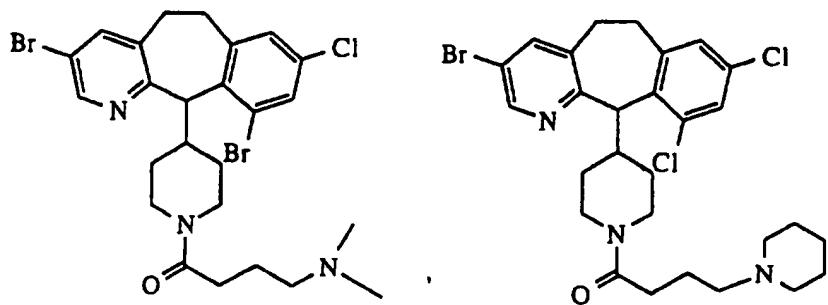
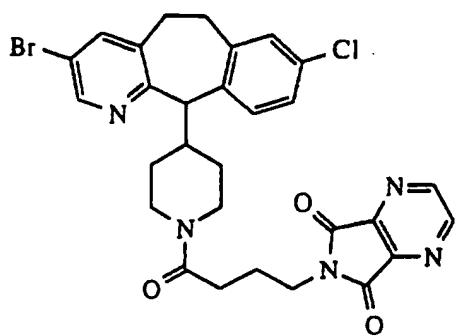
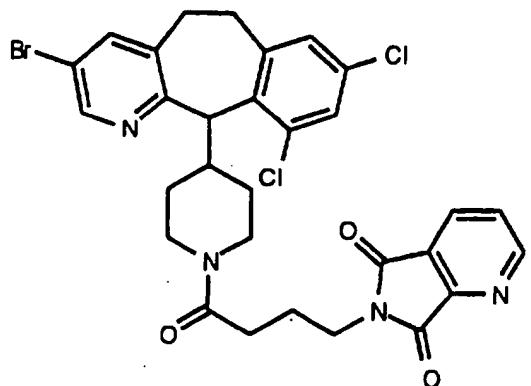
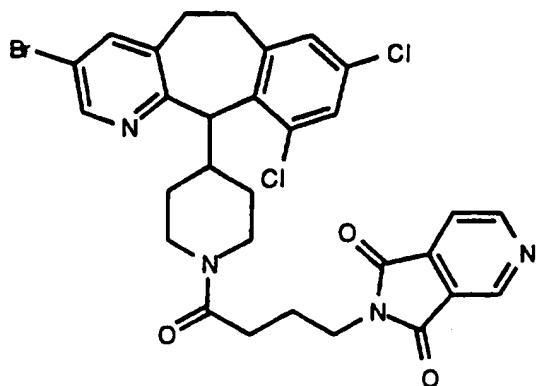
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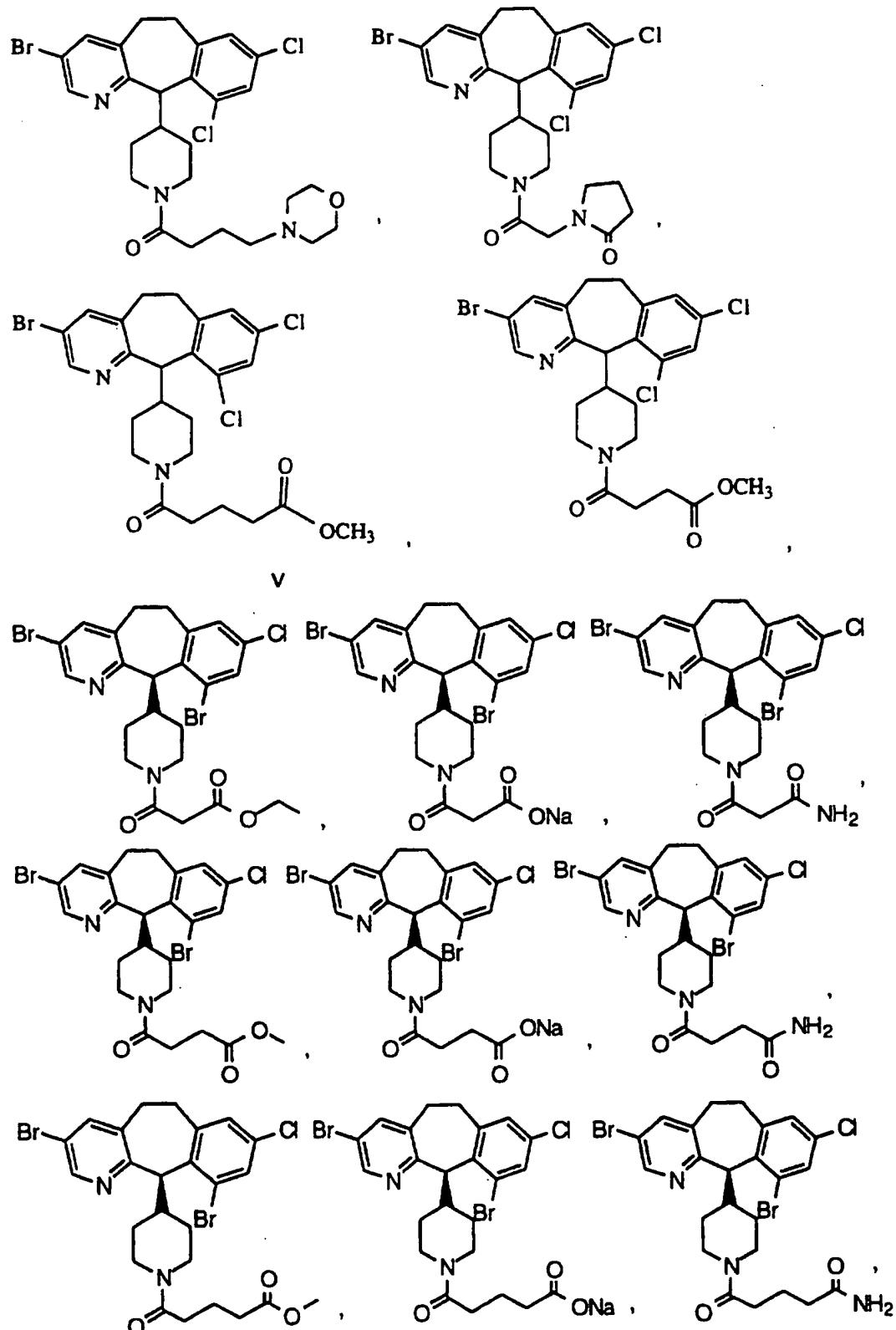
-78-



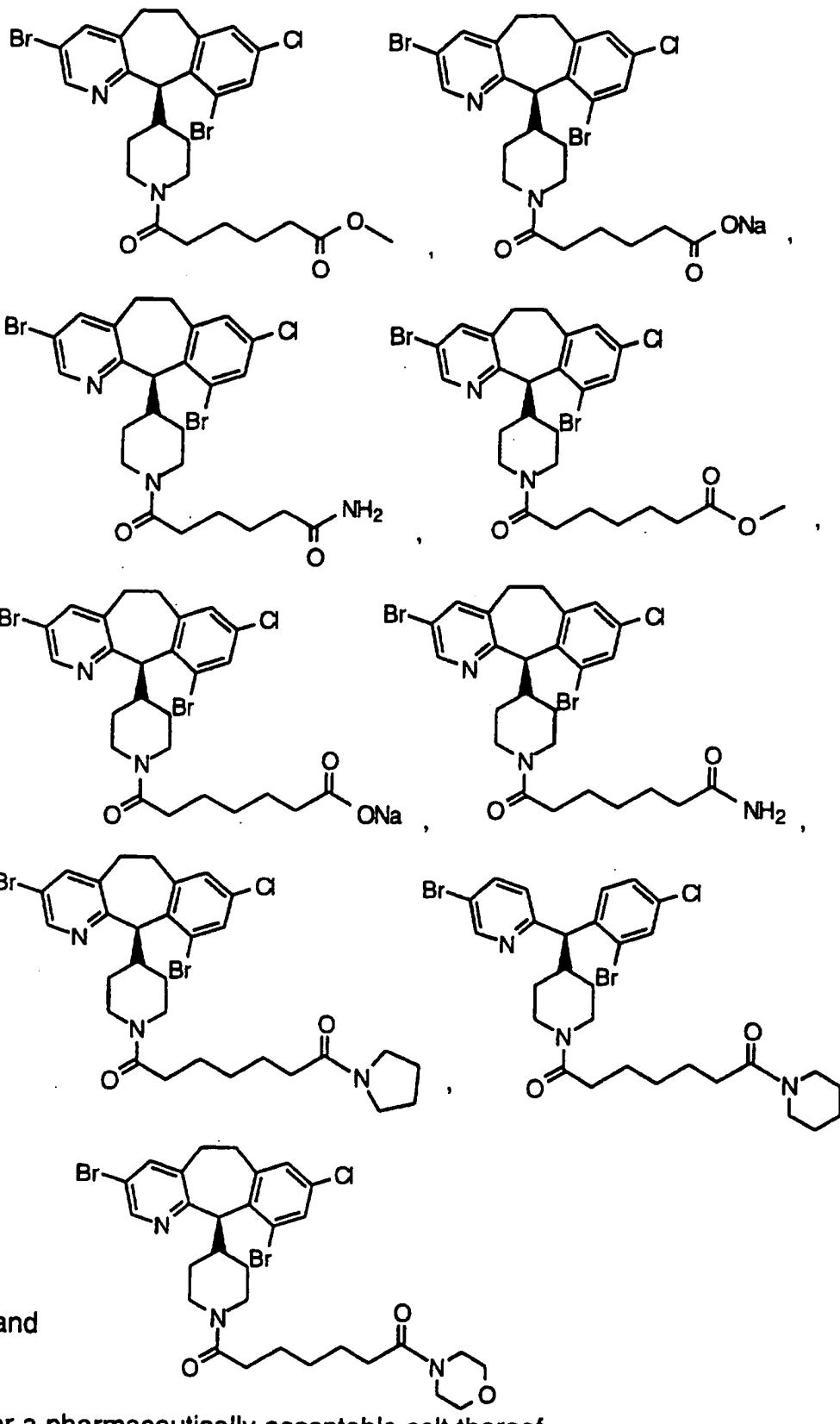
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9. A method of treating tumor cells expressing an activated ras oncogene comprising administering an effective amount of a compound of claim 1.

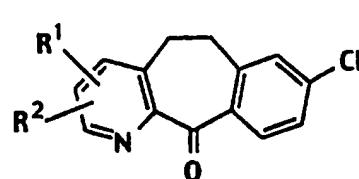
5 10. The method of claim 9 wherein the cells treated are pancreatic tumor cells, breast cancer cells, prostate cancer cells, lung cancer cells, myeloid leukemia tumor cells, thyroid follicular tumor cells, myelodysplastic tumor cells, epidermal carcinoma tumor cells, bladder carcinoma tumor cells or colon tumors cells.

10 11. The method of claim 9 wherein the inhibition is of tumor cells wherein the Ras protein is activated as a result of oncogenic mutation in genes other than the Ras gene.

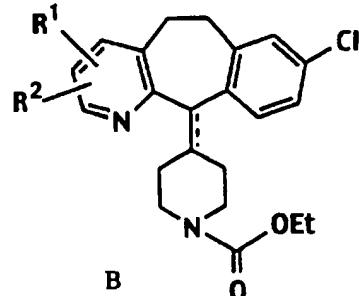
15 12. A method of inhibiting farnesyl protein transferase comprising the administration of an effective amount of the compound of claim 1.

13. A pharmaceutical composition for inhibiting the abnormal growth of cells comprising an effective amount of compound of claim 1 in combination with a pharmaceutically acceptable carrier.

20 14. A process for converting a compound of the formula



or



to the corresponding 7- or 9- nitro compound, which comprises:

25 a) combining a compound of formula A or B with an aqueous acid,
b) cooling the reaction mixture to a temperature in the range of -20°C to -40°C,
c) and then adding KNO₃ at the temperature of step b.

30

INTERNATIONAL SEARCH REPORT

Internat... al Application No

PCT/US 97/15899

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C07D401/04 A61K31/55 C07D221/16 C07D401/12
 //((C07D401/04,213:00,207:00),(C07D401/04,213:00,213:00),
 (C07D401/04,213:00,209:00))

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 10516 A (SCHERING CORP) 20 April 1995 cited in the application see the whole document ---	1-7, 12, 13
X	WO 95 10515 A (SCHERING CORP) 20 April 1995 see the whole document ---	1-7, 12, 13
X	EP 0 495 484 A (SS PHARMACEUTICAL CO) 22 July 1992 see the whole document ---	1-7, 12, 13
X	EP 0 270 818 A (SCHERING CORP) 15 June 1988 see the whole document ---	1-7, 12, 13
		-/-

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *&* document member of the same patent family

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Date of the actual completion of the international search

4 February 1998

Date of mailing of the international search report

20.02.98

Name and mailing address of the ISA

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Authorized officer

Stellmach, J

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 97/15899

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP 0 396 083 A (SCHERING CORP) 7 November 1990 see the whole document ---	1-7,12, 13
Y	BISHOP,W.R. ET AL.: "Novel Tricyclic Inhibitors of Farnesyl Protein Tranferase" J.BIOL.CHEM., vol. 270, no. 51, 22 December 1995, ROCKVILLE PIKE, pages 30611-30618, XP002050604 see the whole document ---	1-7,12, 13
X,P	WO 96 30363 A (SCHERING CORP) 3 October 1996 see the whole document ---	1-7,12, 13
X,P	WO 96 30362 A (SCHERING CORP) 3 October 1996 see the whole document ---	1-7,12, 13
X,P	WO 96 31478 A (SCHERING CORP ;PHARMACOPEIA INC (US)) 10 October 1996 see the whole document ---	1-7,12, 13
X,P	WO 96 30018 A (SCHERING CORP) 3 October 1996 see the whole document ---	1-7,12, 13
X,P	WO 96 31477 A (SCHERING CORP) 10 October 1996 see the whole document ---	1-7,12, 13
Y,P	WO 97 23478 A (SCHERING CORP) 3 July 1997 cited in the application see the whole document ---	1-7,12, 13
X,P	NJORGE,F.G. ET AL.: "Novel Tricyclic Aminoacetyl and Sulfonamide Inhibitors of RAS Farnesyl Protein Transferase" BIOORG.&MED.CHEM.LETT., vol. 6, no. 24, December 1996, OXFORD, pages 2977-2982, XP002054541 * see page 1978, scheme 1, LORATADINE and page 2979, table 1 * see the whole document -----	1-7,12, 13

INTERNATIONAL SEARCH REPORT

International application No.

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Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: 8-11 because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 8-11 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern. Appl. No.

PCT/US 97/15899

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9510516 A	20-04-95	AU 7970394 A CA 2174104 A EP 0723540 A HU 76056 A JP 8510760 T US 5696121 A ZA 9407971 A	04-05-95 20-04-95 31-07-96 30-06-97 12-11-96 09-12-97 12-07-96

WO 9510515 A	20-04-95	AU 7930994 A CA 2174105 A EP 0723538 A HU 76066 A JP 8510759 T ZA 9407970 A	04-05-95 20-04-95 31-07-96 30-06-97 12-11-96 12-07-96

EP 0495484 A	22-07-92	CA 2059363 A JP 1954463 C JP 5059040 A JP 6076403 B US 5231101 A	19-07-92 28-07-95 09-03-93 28-09-94 27-07-93

EP 0270818 A	15-06-88	US 4826853 A AT 116310 T AU 635400 B AU 7285991 A AU 604285 B AU 8336287 A CA 1305147 A CA 1321589 A CS 9104143 A DE 3750929 D DE 3750929 T DK 73193 A DK 354688 A EP 0330673 A EP 0685476 A ES 2068179 T FI 96768 B HK 186396 A IE 65174 B	02-05-89 15-01-95 18-03-93 30-05-91 13-12-90 25-05-88 14-07-92 24-08-93 16-09-92 09-02-95 01-06-95 21-06-93 28-06-88 06-09-89 06-12-95 16-04-95 15-05-96 11-10-96 04-10-95

INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern. Appl. No.

PCT/US 97/15899

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0270818 A		JP 6078316 B JP 2500910 T OA 9546 A PH 26184 A WO 8803138 A US 5089496 A US 5665726 A US 5438062 A ZA 8708128 A	05-10-94 29-03-90 31-01-93 18-03-92 05-05-88 18-02-92 09-09-97 01-08-95 29-04-88
EP 0396083 A	07-11-90	US 5089496 A AT 114650 T AU 643946 B AU 5642090 A CA 2053903 A DE 69014393 D DE 69014393 T EP 0471750 A ES 2064520 T IE 66392 B IL 94258 A JP 4504724 T KR 9509859 B NO 179674 B OA 9521 A WO 9013548 A US 5151423 A US 5438062 A	18-02-92 15-12-94 02-12-93 29-11-90 02-11-90 12-01-95 01-06-95 26-02-92 01-02-95 27-12-95 07-10-94 20-08-92 29-08-95 19-08-96 15-11-92 15-11-90 29-09-92 01-08-95
WO 9630363 A	03-10-96	AU 5307796 A EP 0815100 A US 5696121 A	16-10-96 07-01-98 09-12-97
WO 9630362 A	03-10-96	US 5700806 A AU 5307696 A EP 0815099 A	23-12-97 16-10-96 07-01-98
WO 9631478 A	10-10-96	AU 5527996 A EP 0819121 A NO 974610 A	23-10-96 21-01-98 08-12-97

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 97/15899

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9630018 A	03-10-96	AU 5189196 A EP 0817632 A	16-10-96 14-01-98
WO 9631477 A	10-10-96	AU 5432896 A EP 0819120 A US 5672611 A	23-10-96 21-01-98 30-09-97
WO 9723478 A	03-07-97	AU 1331197 A	17-07-97